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THE FISHES OF NORTH CAROLINA'S BAY LAKES AND THEIR INTRASPECIFIC VARIATION[1]

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In 1942 Fowler described a new species of minnow (*Notropis waccamanus*) from Lake Waccamaw in North Carolina, and four years later Hubbs and Raney described three more new species of fish from this lake (*Fundulus waccamensis*, *Boleosoma perlongum*, and *Menidia extensa*). These four species were regarded as endemics that had differentiated within the lake itself.

In 1947 a survey of the bay lakes (of which Waccamaw is one) was made under the author's direction for the North Carolina Wildlife Resources Commission, at which time rather extensive collections of the fishes in these lakes were made. Only a preliminary paper on this material (Hueske, 1948) has been published, in which a number of the species present were not recognized or are listed only as to genus, and in which there is no description of the individual species.

Since there is no good reason at present for believing that Lake Waccamaw is any older than the bay lakes in Bladen County, there is the possibility that certain of the species in these lakes may likewise exhibit differentiation. Accordingly the survey collection was studied in considerable detail to determine the characteristics of the various populations, the variation from one lake to another, and significant differences from neighboring populations.

The Lakes

The morphometry and chemistry of the lakes concerned in this study have already been reported on in detail (Frey, 1949). Five of them (Jones, Salters, Singletary, White, and Black, in order of increasing size) are located in Bladen County, and the sixth (Waccamaw) is in adjacent Columbus County. The area

[1] Contribution No. 468 Department of Zoology, Indiana University. The author is indebted to E. E. Hueske for making his field notes available, to the North Carolina Wildlife Resources Commission for various courtesies, and to Drs. R. M. Bailey and R. R. Miller of the University of Michigan Museum of Zoology for helpful advice and the use of the Museum collections. Completion of this report was made possible through a research grant from Indiana University.

of these lakes varies from 224 acres in Jones to 8938 acres in Waccamaw. All the lakes are less than 12 feet deep, and there is no evidence that they have been exceptionally deep at any time in their history (Frey, 1950).

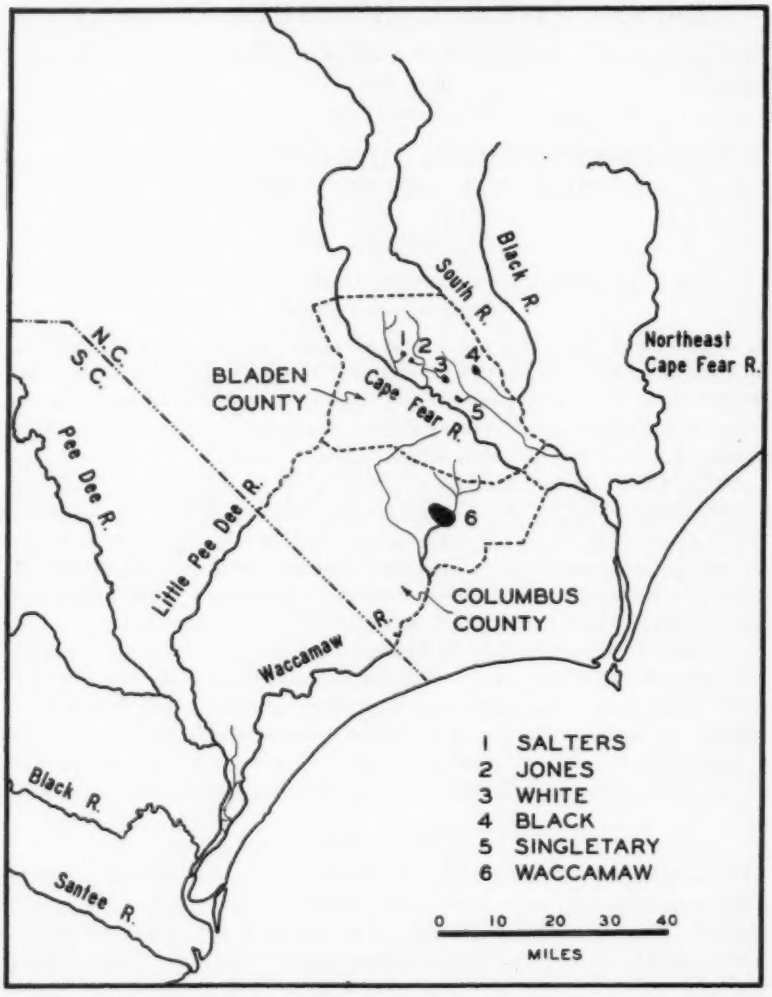

FIG. 1. Location of the bay lakes of North Carolina with respect to their drainage connections.

Waccamaw has an almost neutral chemical reaction, but the rest are strongly acid, with the pH varying from 4.3 in Jones Lake to 4.9 in White Lake. The water in White Lake is virtually colorless (<10 ppm.), whereas that in the other

lakes has a brown color ranging from 160–180 ppm. in Waccamaw, Singletary, and Black to 300 ppm. in Jones and Salters. Calcium content is very low, and the lakes in general are not very productive. Waccamaw shows by far the greatest production of fish and benthos. Next in order of decreasing productivity come White, Singletary, Jones, Black, and Salters. Being shallow and large in area the lakes exhibit only temporary thermal stratifications. The oxygen content of the water is undoubtedly always adequate for the fishes present, although it was never up to saturation during the period of examination.

All the lakes have surface outlets. In the Bladen County lakes these outlets are small and shallow and may be intermittent during exceptionally dry periods. The outlet of Waccamaw is much larger. Before the present concrete dam was constructed in its outlet, the lake was probably accessible to river fish at all times with the exception of extremely low water levels. Singletary Lake likewise has a spillway dam at the outlet that probably restricts access of fish to the extreme high water periods. Except as mentioned above, the lakes are not isolated at the present time: there is the possibility of exchange between the river populations and the lake populations.

The lakes in Bladen County are all located in the Cape Fear River Basin (Fig. 1). The creeks coming from the lakes flow through extensive swampy areas (other bays) in tortuous channels with little gradient. The rate of water movement is normally slow, and the water has a dark brown color. Salters Lake connects with the Cape Fear River near Elizabethtown via Ellis Creek. Jones and White lakes both drain into Turnbull Creek, which enters the Cape Fear just below Elizabethtown. Singletary and Black lakes after quite a few miles of swampy channels drain into Black River, which is one of the major tributaries of the Cape Fear, joining the parent stream about 10 miles north of Wilmington. Hence, the Bladen County lakes are divisible into 2 groups according to drainage pattern: Salters, Jones, and White Lakes in one group, and Black and Singletary in the other. This dichotomy in drainage helps to explain the present distribution of at least 2 species of fish.

Waccamaw, on the other hand, is in an entirely different drainage system. The Waccamaw River arising from the lake and from the large swamp north of the lake flows southward into South Carolina, roughly paralleling the coast, finally anastomosing in several channels with the larger Pee Dee River before reaching the ocean near Georgetown, South Carolina.

AGE OF THE LAKES

The age of these lakes, and of the Carolina bays in general, is not definitely known. Estimates are variously given from the Pleistocene to late Cretaceous (Richards, 1950). Cooke (Hubbs and Raney, 1946) argues that the site of Lake Waccamaw was covered by the sea during all of Sangamon time, and that therefore this lake could not have existed prior to the last quarter of the Pleistocene.

More recently the author has made some studies on the sediments of Singletary Lake (Frey, 1951). In this lake there are three layers of organic matter, of which the radiocarbon age of the middle layer, as determined by the Institute of Nuclear Studies at the University of Chicago (Arnold and Libby, 1951), is

greater than 20,000 years, and the age of the beech-hickory-oak maximum in post-glacial time is approximately 10,000 years. Assuming constant rates of sedimentation, the age of the lowermost sediments is estimated between 40,000 and 100,000 years, which would be well back in Wisconsin time, and might even extend into the Sangamon interglacial age.

There is no evidence from the examination of the bottom sediments that the lake was ever completely dry during this long interval of time. It is quite likely, however, that it and the other bay lakes have been isolated from their drainage channels during at least two periods when the climate was drier than at present. If conditions in the lakes were suitable for maintaining fishes during these times, and if the periods of isolation were long enough, genetic differentiation and reproductive isolation of some of the populations might be expected. Very likely this is what has happened in Lake Waccamaw.

METHODS

Each lake was worked intensively for two weeks with seines, dip nets, gill nets, trammel nets, fly rod, pole, and rotenone. The specimens collected were killed in formalin, and after sufficient hardening transferred to 70 per cent alcohol. One cannot be certain that all species present in the lakes are represented in the collections, although it is reasonably certain that all the major populations were sampled. Since 9 out of the total of 85 species records for the lakes are based on just single specimens, it is likely that more extensive collecting would have disclosed other small populations, particularly of predators, in some of the lakes.

Some specimens from this collection have been deposited in the museum collections at the University of Michigan and at Cornell University. The bulk of the collection is in the Department of Zoology at North Carolina State College, Raleigh.

All measurements and counts on the specimens were made according to the descriptions in Hubbs and Lagler (1947). Measurements were made with a vernier caliper and all proportions reported in the tables were calculated from these measurements. The proportional measurements in the tables are in parts per thousand of the standard length. Counts of the dorsal and anal fin rays in the esocids and ameiurids are total counts based on dissection. Counts in the other two major groups are of the principal rays, counting the last two rays as one. The only exception to this procedure was in *Fundulus dispar lineolatus*, where the last two rays were counted separately because they evidenced little or no approximation at their bases.

VARIATION

Fish systematists habitually employ various body proportions and meristic counts in describing species and lesser taxonomic categories, and in defining the differences between such categories that are closely related. The basic assumption involved in the taxonomic use of these characters is that they are controlled primarily by heredity. It is becoming increasingly apparent as a

result of recent work, however, that both meristic counts and proportional measurements can be considerably modified by environmental influences. An understanding of the nature and causes of variability is therefore of fundamental importance.

Every population is variable, both in continuous and discontinuous characters. Part of this variability is the result of factors acting on each individual separately, such as the extent of heterozygosity and the effect of environmental fluctuations during certain critical periods in ontogeny. Gabriel (1944), for example, found in *Fundulus heteroclitus* that the vertebral counts of separately reared groups of sibling offspring varied, even though environmental conditions (temperature) were kept constant. Gosline (1947) found rather extensive variation in meristic characters correlated positively with size of individuals in an entire year class of darters collected at one time. He assumed that the largest individuals developed from the eggs laid earliest when the water temperatures were the coldest, and that therefore the variation in meristic characters with size of fish was really an inverse temperature series. These are two examples of what is frequently termed the "normal variation" in populations.

Another component of the variability present in populations is that arising from differential growth or heterauxesis of the various body parts with respect to one another and to the body as a whole. Thus, in many of the species included in this report the head and eye usually increased in relative size with increasing length of fish, and the depth of body decreased. It has been demonstrated recently (Martin, 1949) that the rate of change in relative size of these various body parts is constant for each individual over extensive length ranges or stanzas, and that changes in rate occur rather abruptly (inflections in the logarithmic curves) at certain critical points in ontogeny. Moreover, the slope of the curves (i.e. the rate of change in relative size) within a particular growth stanza appears to be characteristic of the species. Differences in body proportions between different populations of the same species result primarily from variation in mean size of individuals at the times of the several critical periods. Such heterauxetic changes hamper the comparison of populations, especially when only small samples are available for study.

Comparison of two different populations involves the problem of attempting to interpret the significance of differences between them. There is more to it than merely calculating a statistical test of significance. The fishery biologist may be interested in these differences in following migrations of populations or in helping to recognize local races, without being too much concerned as to whether the differences are primarily hereditary or environmental. The systematist, on the other hand, is interested mainly in the genetic differences. Naturally any environmental agents that increase the variability or shift the range of variation of the populations are going to confound the studies of the systematist.

It has been recognized for almost a century that in a species with an extensive and continuous north-south range there may be progressive differences or clines in certain meristic characters. Decrease in number of vertebrae in fishes towards the tropics is almost universal, and in addition there may be clines in other

characters, such as scales and fin rays. Jordan (1891) supposed that such meristic differences were entirely the result of natural selection (and hence were genetic). More recently systematists have concluded that because such meristic gradients are apparently correlated with environment (temperature as related to latitude) they are probably determined by the environment. (See Schultz, 1927, for a typical example of this line of reasoning. Hubbs, 1934, reviews the literature in the field.)

In fairly recent time it has been demonstrated experimentally that the magnitude of certain meristic characters can be influenced by environmental conditions. Mottley (1937) found an inverse relationship between temperature during development and the number of vertebrae in *Salmo*. Gabriel (1944) noted a similar relationship in *Fundulus*, although some batches of "temperature stabile" eggs yielded high vertebral counts even at warm temperatures. Tåning (1946) working on the sea-trout found a sensitive period in development at 145–165 day-degrees, during which time, depending on the temperature, differences up to 1.5 vertebrae could be obtained between sibling lots. The number of vertebrae is fixed well before hatching.

With respect to other meristic characters, Mottley (1934) in some exploratory experiments noted a rather pronounced inverse correlation between temperature during development and the number of scales along the longitudinal axis of trout. Tåning (1946) recorded that the number of fin rays in the dorsal, anal, and pectoral fins of the sea-trout is likewise determined prior to hatching, but he made no mention of their being influenced by environmental conditions. Finally, as to other environmental influents, Tåning stated that oxygen tension has a small inverse effect on the number of vertebrae in the sea-trout. The current understanding of the whole problem of variation in meristic characters of fishes is that although the characters are primarily genetic, they can be modified one way or another in significant degree by various environmental factors prior to hatching.

Martin (1949) generalized that both meristic characters and proportional sizes tend to vary with rate of development: the number of meristic parts is greater the slower the rate of development, whereas the size of such body parts as head and fins exhibits a direct relationship to rate of development. Temperature and oxygen tension are just two of a number of factors that conceivably could affect rate of development.

If the characters used taxonomically can vary according to environmental conditions prevailing during these critical periods prior to hatching or even shortly after hatching, then one might expect population differences in these characters to occur from one year class to another in the same lake, and from one lake to another within the same general region, depending on the temperature regimes of the several years, the morphometry of the basins (as controlling temperature), and possibly the chemistry of the waters. Where the differences between two populations are beyond the range of normal variation and are neither accounted for by heterauxesis nor correlated with any discernible en-

vironmental factor or complex, then the populations are probably genetically distinct. Where these differences are great enough, according to accepted standards, the populations can be given separate taxonomic recognition. For all practical purposes, however, it is usually virtually impossible to separate the effects of environment and heredity.

In the present study attempts are made to discover and describe the differences in species characters from one lake to another, and in certain instances to determine the extent of differences between the lake populations and neighboring populations.

RESULTS

Table 1 shows the number of specimens of each species caught during the survey of the lakes, as nearly as can be ascertained from the field notes. The adjacent figures in parentheses represent the actual number of specimens that were in the collection and were examined. These latter figures include a subsequent collection by the author each in Singletary, Waccamaw, and White lakes. Apart from differences arising from this cause, other discrepancies between the two sets of figures represent in part specimens that have been transferred to the fish collection at Cornell University under the direction of E. C. Raney.

In quite a number of instances there are present adequate series of fingerlings and adults for a study of the body proportions and meristic characters of the populations. These are discussed in the following pages under the individual species.

Lepisosteus osseus osseus (Linnaeus)
Eastern longnose gar

The two specimens in the collection are 660 and 600 mm. standard length. Four others caught during the survey ranged from 280 to 690 mm. Proportional measurements: head 318, 315; body depth 98, 77; eye diameter 24, 24; snout length 218, 213; least snout width 14, 12. Counts: scale rows 59; dorsal rays 8, 7; anal rays 9, 8; pectoral rays 10, 12; pelvic rays 6, 6. These figures are well within the range of variation of the longnose gar. The two specimens, particularly the larger one, exhibits the roughening of the scales on the anterior part of the body said to be typical of the subspecies *osseus* along the Atlantic Coast (Hubbs and Allen, 1943). In the group of lakes under consideration the species occurs only in Waccamaw.

Amia calva Linnaeus
Bowfin

This species, known locally as blackfish, is represented in the collection by two specimens from White Lake 280 and 374 mm. standard length, and a single specimen from Waccamaw 342 mm. Another specimen from Waccamaw weighing 11½ lbs. was caught in a trammel net. This specimen is near the upper size limit for the species, as reported by Smith (1907).

TABLE 1

Distribution of species by lake and number of specimens collected during the survey. The figures in parentheses are the numbers of specimens actually examined in the present study.

SPECIES	JONES	SALTERS	SINGLETARY	WHITE	BLACK	WACCAMAW
Lepisosteus osseus osseus						6 (2)
Amia calva				3 (2)		2 (1)
Dorosoma cepedianum						7 (7)
Esox americanus	1 (1)	2 (2)	2 (2)	1 (1)	2 (0)	
Esox niger	2 (2)		2 (2)	3 (1)		5 (0)
Erimyzon sucetta sucetta	39 (18)	5 (5)	579 (422)	25 (5)	19 (8)	
Erimyzon oblongus						1 (1)
Notemigonus crysoleucas bosci						203 (121)
Notropis chalybaeus			391 (282)			
Notropis petersoni						119 (119)
Ictalurus catus						38 (23)
Ameiurus natalis	13 (12)	21 (9)	97 (13)	119 (26)	22 (6)	
Schilbeodes mollis	40 (30)	10 (10)	28 (20)	106 (20)	11 (9)	35 (14)
Anguilla rostrata				*		4 (4)
Fundulus waccamensis						0 (12)
Fundulus dispar lineolatus	9 (4)	62 (60)		24 (14)		
Gambusia affinis holbrooki			5 (5)	46 (27)		23 (26)
Menidia extensa						5 (2)
Morone americana						99 (58)
Micropterus salmoides salmoides	1 (0)	1 (1)		34 (21)		22 (10)
Chaenobryttus coronarius	8 (7)	9 (9)	227 (206)	35 (15)	6 (7)	15 (8)
Lepomis punctatus						0 (2)
Lepomis gibbosus						79 (86)
Lepomis auritus				4 (2)		30 (32)
Lepomis macrochirus purpurescens			1 (0)	10 (3)		302 (225)
Enneacanthus gloriosus	53 (29)	60 (60)	157 (198)	35 (18)	99 (85)	5 (3)
Centrarchus macropterus	7 (7)	1 (1)	47 (51)	1 (1)	2 (1)	
Pomoxis nigro-maculatus						26 (19)

TABLE 1.—(*Continued*)

SPECIES	JONES	SALTERS	SINGLETARY	WHITE	BLACK	WACCAMAW
Perca flavescens	39 (30)	7 (7)	18 (21)	320 (376)	35 (15)	48 (154)
Boleosoma perlongum						(6)
Hololepis thermophilus thermophilus				254 (34)		66 (8)
Hololepis thermophilus n. subsp.	87 (96)	? (50)	93 (33)			
Total number of species.	12	11	13	17	8	25

* One specimen observed during night collecting.

Dorosoma cepedianum (LeSueur)
Gizzard shad

Occurring only in Lake Waccamaw the gizzard shad is represented in the collection by 7 specimens, falling into two distinct size groups. The 4 small specimens have a length range of 173–189 mm., with a mean of 183, whereas the 3 large specimens are 246–294 mm. long, with a mean of 267.

Between these two groups there are marked differences in relative depth of body and size of head, indicating that as the fish increase in length the body becomes deeper and the head smaller. Body depth averaged 363 (range 354–371) in the small specimens, and 388 (range 380–392) in the large specimens. Head length averaged 325 (range 318–336) in the small specimens, and 295 (range 291–302) in the large specimens. Counts: longitudinal scales, 52–58, av. 54.4 (5); transverse scales, 19–22, av. 21.0 (5); dorsal rays 13–15, av. 14.3 (7); anal rays 30–34, av. 32.0 (7). The longitudinal scale count is below the range of 56–65 listed by Smith (1907), and the dorsal ray count is somewhat greater. In the present study all rays are included in the counts.

Esox americanus Gmelin
Redfin pickerel
(Table 2)

This smaller species of *Esox* occurs in all the Bladen County lakes but was not collected in Lake Waccamaw. It may possibly occur in this lake, however, since it is widely distributed in the coastal region of North Carolina.

The eye of this specimen is noticeably closer to the snout than to the edge of the operculum. The color pattern varies considerably. On the sides is a series of rather broad vertical bars, sloping forward ventrally, and either terminating before the belly is reached, or extending all the way to the ventral side, in which case the belly has a mottled appearance. The coloration beneath the head varies from almost none to a distinct mottling, with 2 pronounced longitudinal stripes and various spots anterior to the branchiostegals. The characters for this species are listed in Table 2, along with those for *E. niger*.

Esox niger LeSueur
Chain pickerel
(Table 2)

Specimens of this species were collected in all the lakes except Salters and Black. The largest specimen, from Waccamaw, was 461 mm. standard length and weighed 884 g. Two specimens from Singletary were almost as large. These large specimens are mottled with more or less separate series of irregular vertical blotches above and below the lateral line. In this species the eye is closer to the posterior edge of the operculum than to the snout.

Esox niger and *E. americanus* can be distinguished by the number of branchiostegals, dorsal and anal rays, and scales, as indicated in Table 2, as well as by the eye position.

TABLE 2

Proportional measurements and meristic counts in Esox americanus *and* Esox niger *from the bay lakes*

	E. AMERICANUS			E. NIGER		
	No.	Range	Mean	No.	Range	Mean
Standard length (mm.)	6	115–152	138	5	53.7–430	214
Head length	6	319–343	332	5	325–362	343
Body depth	6	165–215	180	5	133–192	159
Eye diameter	6	57–68	60	3	62–63	63
Rays: dorsal	6	16–18	17.3	5	19–20	19.6
Rays: anal	6	15–16	15.5	5	17–18	17.6
Branchiostegals	6	12–13	12.7	5	14–16	15.4
Longitudinal scales	5	94–107	99.4	1		118

Erimyzon sucetta sucetta (Lacépède)
Eastern lake chubsucker
(Tables 3, 4; Fig. 2)

All the Bladen County lakes have this species represented. Tables 3 and 4 show that the range of variation of these populations is closer to that of subspecies *sucetta* than to that of the western subspecies *kennerlyi*, as given by Hubbs (1930). The number of rays in the dorsal fin is usually 12 or more, rather than fewer, and the number of scales in longitudinal series to the end of the caudal peduncle is most commonly 35 or 36, rather than 36–38.

One feature of these populations that does not seem to be typical of the species is the presence of more than 3 breeding tubercles on the head, as shown in Fig. 2. Hubbs (1930) previously reported a single male from Mobile with several smaller accessory tubercles in addition to the three primary ones, but in the present populations all the tubercle-bearing specimens have more than the typical 3, at least on one side of the head. The specimen from White Lake illustrated had 5 tubercles on each side, all in front of the eye. Another specimen from White Lake, 320 mm. standard length, had 22 tubercles on the left side and 16 on the

right side. These included 12 and 4 tubercles, respectively, below the eye. The specimen from Black Lake in Fig. 2 shows a general pattern of distribution of these supernumerary tubercles. What appears to have happened is that each of the 3 primary tubercles has broken up into a number of smaller tubercles, and that additional tubercles have developed in this same area, in the region below the eye, and to a lesser extent even above the eye or on the bones of the opercu-

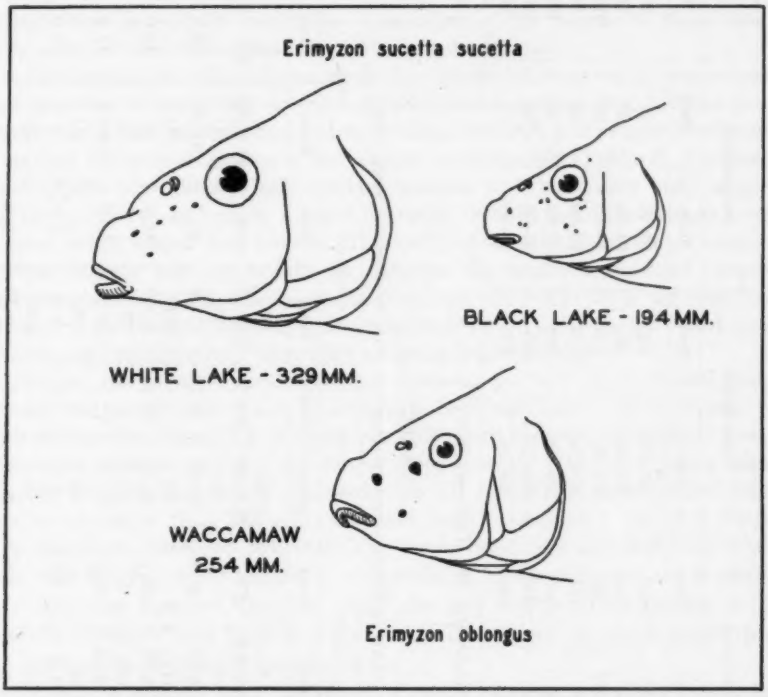

FIG. 2. Distribution of breeding tubercles in *Erimyzon*. The typical condition in the genus is three tubercles, as shown in *Erimyzon oblongus* from Waccamaw. All specimens of *Erimyzon sucetta* from the Bladen County lakes had supernumerary tubercles on at least one side of the head. The illustrated specimens from White and Black lakes show some of the extreme variability in this character.

lum. Development of these accessory tubercles may possibly be correlated with the large average size of the specimens or may be a population characteristic.

The number of tubercles is not constant for any lake, as already mentioned for the White Lake material. Two other tuberculate specimens from Black Lake, 194 and 205 mm. standard length, had 5 or 6 tubercles on each side of the head, much as in the illustrated White Lake specimen. Of two tuberculate specimens from Jones Lake, 237 and 252 mm. long, the former had 9 tubercles on the

TABLE 3

Changes in proportional measurements with increasing size in Erimyzon sucetta sucetta *from the Bladen County lakes*[1]

STANDARD LENGTH (MM.)	NO.	BODY DEPTH		HEAD LENGTH		EYE DIAMETER		WIDTH BONY INTERORBITAL	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
25–49	14	242–283	260	280–309	296	67–95	77	89–104	99
50–74	11	250–281	264	258–295	283	64–76	69	90–100	95
75–99	2	274–299	287	275–282	279	59–63	61	102–105	104
150–199	4	293–327	314	259–287	273	50–55	53	99–111	106
200–249	7	292–334	316	269–300	280	48–60	55	102–114	110
250–299	3	280–328	306	278–302	289	51–58	53	106–126	113
>300	2	323–342	333	273–280	277	46–48	47	98–107	103

[1] Frequencies: Jones—8; Salters—5; Singletary—20; White—4; Black—7.

TABLE 4

Meristic counts in Erimyzon sucetta sucetta *from the Bladen County lakes*

	JONES		SALTERS		SINGLETARY		WHITE		BLACK	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
	(8)		(5)		(38)		(4)		(7)	
Standard length (mm.)	25.9–253	279	36.0–199	69	33.5–251	69	56.4–329	191	43.3–203	133
	(8)		(3)		(23)		(4)		(6)	
Scales: longitudinal	36–37	36.3	35–36	35.7	35–38	36.7	35–37	35.8	35–37	36.0
	(7)		(3)		(12)		(4)		(3)	
Scales: transverse[1]	15–17	16.1	16–17	16.7	15–17	16.4	15	15.0	17	17.0
	(8)		(5)		(38)		(4)		(7)	
Rays: dorsal	12–13	12.5	12–13	12.6	11–13	12.0	12–13	12.8	11–13	12.0
	(8)		(5)		(38)		(4)		(7)	
Rays: anal	7–8	7.1	7	7.0	7–8	7.0	7	7.0	7–8	7.3
	(8)		(5)		(33)		(4)		(7)	
Rays: pelvic	9	9.0	9–10	9.6	9–10	9.2	9	9.0	9–10	9.7

[1] These counts do not include the median dorsal and median ventral scales.

left side and 15 on the right, the latter had 8 tubercles on each side. A 209 mm. specimen from Singletary Lake had 8 small tubercles on the left, but only the 3 typical tubercles on the right. Another specimen 214 mm. long had 6 tubercles on the left and 5 on the right. No specimens from Salters Lake had tubercles. These examples demonstrate the great variability in number and distribution of the tubercles. Any possible taxonomic significance of these tubercles is not known, although their constant occurrence may indicate that the lake populations are differentiated genetically from other populations of the region. All these specimens also had the bilobed anal fin profusely tuberculated.

In the small series of specimens studied no marked differences in proportional measurements between the various populations could be discovered. When these measurements are tabulated according to size, however, it is evident that there is marked differential growth of the several parts studied (Table 3). The body depth shows a progressive and marked increase with increasing body length, and the eye shows an equally marked decrease. In both series the change is not so great in fish longer than 150 mm. The width of the bony interorbital tends to increase slightly with increase in size, whereas the length of the head remains approximately constant relative to the length of the body. Thus the head:eye ratios used by Hubbs (1930) in separating *sucetta* from *oblongus* are based upon a decreasing eye diameter, rather than an increasing head length.

Likewise the meristic counts do not demonstrate any marked differences between the populations (Table 4), although the White Lake population may be distinct from the others. The 3 specimens studied all had only 15 scales in transverse series, whereas only 2 of the 26 specimens from the other lakes had a count this low. In addition the fish captured with gill nets in the dark-colored lakes were never longer than 268 mm. standard length, whereas 3 out of 7 White Lake specimens exceeded this size. Two males were 339 and 340 mm. when fresh, with weights of 938 and 984 g., respectively. The populations are so nearly alike otherwise, however, that this larger size may merely be a reflection of the generally better trophic level of White Lake. These sizes are much larger than any reported by Hubbs for the species.

Immature specimens from the Bladen County lakes agree well in their color pattern. Specimens 60 mm. and less have a solid dark brown lateral stripe about 2 scale rows wide, usually ending in a somewhat broader and considerably darker tail spot just beyond the edge of the caudal peduncle. The stripe is continued in front of the eye where it dips down slightly and thins out near the tip of the snout. Below the stripe the body is almost whitish in color. This region contains a variable amount of pigment, from almost none in White Lake specimens, to outlining of the scales and pigment around the anus and base of the anal fin in others. There may also be scattered chromatophores anterior to the branchiostegals.

Above the lateral stripe the coloration is a dark tan or brownish, with a somewhat lighter stripe immediately above the dark stripe. This lighter stripe continues forward through the nares and tends to form a V-shaped configuration on

the head pointing forward. Between the arms of the V is a rather indistinct light stripe extending from the occiput toward the snout.

The leading edge of the dorsal fin up to and including the first branched ray is heavily pigmented. The next 2 to 5 interradial membranes, depending on the size of the specimen, are largely unpigmented, although there are chromatophores along the rays. The posterior membranes again have chromatophores. The other fins tend to have chromatophores concentrated along the rays.

As the fish increase in size, all the fin membranes become quite generally pigmented, and the lateral stripe becomes less and less distinct, although still clearly visible in specimens as long as 100 mm. The leading edge of the dorsal fin in these larger specimens is not sharply pigmented, and the membranes are generally smoky. The head is more uniformly pigmented, lacking the light V structure and the median stripe.

Erimyzon oblongus (Mitchill)
Creek chubsucker
(Fig. 2)

In the most recent taxonomic paper on this genus (Hubbs, 1930) *Erimyzon oblongus oblongus* was not listed south of Virginia, and *E. oblongus connectens* was not listed north of the Altamaha River in Georgia. This left a big gap, covering South Carolina and North Carolina, in our knowledge of the distribution of this species. Within the past two decades additional series have been added to the collection in the University of Michigan Museum of Zoology. Subspecies *oblongus* is now represented south to the Roanoke River. Subspecies *connectens* is represented by a total of 14 specimens from the upland portion of the Cape Fear system in North Carolina. Nine of these specimens have 11 dorsal rays, the other 5 having 12.

On the basis of the single specimen in the collection it is not possible to tell which subspecies is represented in Lake Waccamaw. Since the Waccamaw River system is south of the Cape Fear, it is likely that the subspecies is *connectens*. The specimen has the typical 3 breeding tubercles, as shown in Fig. 2.

Size: 254 mm. standard length. Proportional measurements: body depth 328; head length 267; eye diameter 54; width bony interorbital 112. Meristic counts: longitudinal scales 41; transverse scales 18; dorsal rays 12; anal rays 7; pelvic rays 10.

Notemigonus crysoleucas bosci (Valenciennes)
Southeastern golden shiner
(Table 5)

Although Hubbs and Raney (1946) were not aware that this species occurs in Lake Waccamaw, the golden shiner is a common forage fish in the lake and probably is quite important in its general economy. Specimens were taken abundantly in many of the rotenone collections.

As is true of other species in this collection, the depth of the body increases and the diameter of the eye decreases with increasing length. There is no change in relative length of head over the size range here represented.

On the basis of the large number of principal anal rays (most commonly 16 or 15) these specimens are placed in the subspecies *bosci*. Other ranges are typical for the species.

The University of Michigan Museum of Zoology has several good series of this subspecies from South Carolina for comparison with the Waccamaw material. The anal ray count on 65 specimens averaged 15.4, which is not appreciably different from the Waccamaw average of 15.8. The number of lateral-line scales on 16 specimens averaged 46.1, which is below that for Waccamaw. Eight specimens from the Waccamaw River in Georgetown Co., S. C. (Museum No. 143250), are almost identical with the Lake Waccamaw specimens in the char-

TABLE 5

Proportional measurements and meristic counts in Notemigonus crysoleucas bosci *from Lake Waccamaw, with comparative material from South Carolina (Univ. Mich. Mus. Zool.)*

	LAKE WACCAMAW		TOTAL SOUTH CAROLINA		WACCAMAW RIVER, S. C.	
	Range	Mean	Range	Mean	Range	Mean
	(24)		(18)		(8)	
Standard length (mm.)	40.1–106	55	57.2–105	79	57.2–79.1	66
	(22)				(8)	
Body depth	218–292	248			241–279	258
	(14)				(8)	
Head length	241–256	249			234–255	248
	(14)				(8)	
Eye diameter	63–76	70			64–75	70
	(21)		(16)		(6)	
Scales: longitudinal	44–56	49.3	43–49	46.1	45–49	46.5
	(18)					
Scales: above lateral line	10–12	10.6				
	(20)					
Scales: below lateral line	4–5	4.6				
	(14)					
Rays: dorsal	7–8	7.9				
	(37)		(65)		(16)	
Rays: anal	14–18	15.8	14–17	15.4	14–17	15.6

acters studied, except that they have a smaller count of lateral-line scales. There is apparently a general tendency for lacustrine populations of many species to develop more scales along the body than their fluviatile counterparts. Hence, this one character is not sufficient to indicate differentiation of the population.

Notropis chalybaeus (Cope)
Ironcolor shiner
(Table 6)

Singletary Lake is the only one besides Waccamaw with a species of cyprinid in its fauna. The species here represented is widely distributed in the coastal

plain of North Carolina and probably represents a fairly recent addition to the fauna of the lake. There are no pronounced differences between this population and others of the region from outside the lake.

In these specimens there is a broad lateral stripe. The lateral line begins at the head end near the upper border of the stripe, then drops to the lower border for a distance about equal to the length of the head, and gradually rises to the middle of the stripe at the tail. In alcoholic specimens there is a faint orange tinge along the anterior fourth of the lateral stripe, as well as an orange spot in the flesh at the base of the tail.

TABLE 6

Proportional measurements and meristic counts in Notropis chalybaeus *from Singletary Lake*

	NO.	RANGE	MEAN ± S.E.
Standard length (mm.)	20	31.1–49.2	38.6
Body depth	14	195–228	206.9 ± 2.36
Head length	14	239–267	253.5 ± 2.62
Eye diameter	14	69–79	73.9 ± 1.01
Scales: longitudinal	20	33–42	36.6 ± 0.58
Scales: above lateral line	18	7–9	8.3 ± 0.14
Scales: below lateral line	19	4–5	4.8 ± 0.12
Lateral-line scales before dorsal	20	11–16	12.8 ± 0.27
Rays: dorsal	20	7–8	8.0
Rays: anal	20	8–9	8.1
Pharyngeal teeth	5		2,4–4,2

Notropis petersoni Fowler
Peterson's shiner
(Table 7)

In the same publication Fowler (1942) described 2 new species of *Notropis* from North Carolina, *Notropis waccamamus* from Lake Waccamaw, and *N. petersoni* from the Cape Fear River. Hubbs and Raney (1946) on the basis of the several specimens available to them suggested that the two species were very closely related and might well be considered as subspecies were it not for apparently sharp differences in color.

In the present study a series of 25 specimens from Lake Waccamaw was compared in detail with the same subtopotypic series of *N. petersoni* from Lakeview, N. C., collected by R. E. Coker, which was used by Hubbs and Raney in their paper, and an additonal series of *petersoni* from Lyons, Ga., collected by R. M. Bailey and M. K. Bailey. The summarized data in Table 7 show that in proportional measurements the chief differences between the Waccamaw series and the other two are that in the former the depth of body is lesser and the length of the caudal peduncle is greater. These differences, however, are not statistically significant. It should be noted, however, that the two series of *petersoni* in the Michigan Museum collection were considerably shrunken, par-

TABLE 7

Proportional measurements and meristic counts in Notropis petersoni *from Lake Waccamaw, compared with a subtopotypic series from Lakeview, N. C., and another series from near Lyons, Ga. (Univ. Mich. Mus. Zool. numbers 70707 and 158072, resp.)*

	LAKE WACCAMAW		LAKEVIEW, N. C.		LYONS, GA.	
	Range	Mean ± s.e.	Range	Mean ± s.e.	Range	Mean ± s.e.
	(25)		(29)		(18)	
Standard length (mm.)	35.7–48.5	38.8	41.6–56.4	50.3	39.4–51.5	45.8
	(25)		(29)		(18)	
Body depth	179–220	200.8 ± 1.86	184–256	209.7 ± 3.11	183–224	205.3 ± 2.56
	(25)		(29)		(18)	
Head length	246–265	255.4 ± 1.12	238–262	252.8 ± 1.05	247–279	259.5 ± 1.92
	(25)		(29)		(18)	
Head depth	142–165	153.0 ± 1.01	146–160	154.0 ± 0.68	149–167	156.9 ± 1.15
	(25)		(29)		(18)	
Eye diameter	69–80	74.1 ± 0.49	67–81	74.0 ± 0.59	69–81	75.9 ± 0.62
	(25)		(29)		(18)	
Peduncle length	230–259	241.8 ± 1.51	209–256	233.8 ± 2.09	213–254	232.4 ± 2.94
	(25)		(29)		(18)	
Peduncle depth	87–98	92.3 ± 0.67	86–100	92.8 ± 0.61	84–102	92.5 ± 1.51
	(24)		(29)		(9)	
Scales: longitudinal	34–37	35.5	34–37	35.6	34–36	35.1
	(24)		(29)		(16)	
Scales: above lateral line	5–6	5.3	5–6	5.4	5–6	5.4
	(24)		(29)		(12)	
Scales: below lateral line	4	4.0	4–5	4.1	4	4.0
	(22)		(29)		(15)	
Lateral line scales before dorsal	11–14	12.1	11–14	12.1	11–12	11.5
	(25)		(29)		(18)	
Rays: dorsal	8	8.0	8	8.0	7–8	7.9
	(25)		(29)		(18)	
Rays: anal	7	7.0	7	7.0	7	7.0
	(3)					
Pharyngeal teeth		2,4–4,2				

ticularly in the belly region. Hence, the body depth of these series as measured is too low, and accordingly there might be a significant difference in this factor. With respect to the peduncle depth, which seemed to be of importance to Hubbs and Raney in characterizing the Waccamaw population, there is no difference in the 3 series.

In meristic counts likewise there are no differences. The distribution of breeding tubercles in the 3 series is the same, namely a small patch in front of each eye continuing as a single row around the front edge of the snout. On the lower jaw they occur in a ∩-shaped series from the tip of the lower jaw back to the eye or even slightly beyond the eye. In coloration the series are virtually identical, except perhaps for the shining lateral band in the Waccamaw population called attention to by Fowler. This is not readily evident in the alcoholic specimens on hand.

Because the Waccamaw population is separable only with great difficulty if at all from populations of *petersoni*, it seems best to reduce *waccamanus* to the synonymy of *petersoni*, inasmuch as the latter name has page priority in Fowler's paper. The minor differences in the lake population may be non-genetic variation of a type apparently present in some of the other species in these lakes.

Ictalurus catus (Linnaeus)
White catfish

The only large ameiurid from Lake Waccamaw represented in the collection and possibly the only species occurring in the lake, is the present species. Although relatively few specimens were collected during the survey, there is evidently quite a large population in the lake. Out of a total of 3950 fish reported by trot-line fishermen during 3 winters (Hubbs and Raney, 1946), 61 per cent were presumably this species. A single overnight trotline set by the survey party on August 20, 1947, yielded 10 catfish on a total of 20 hooks. None of these large specimens was preserved. In the littoral region young catfish were taken sparingly in seines and rotenone collections.

These specimens check well with the description by Jordan and Everman (1896) except that the number of anal rays (obtained by dissection) is greater and the anal fin is longer relative to the body length.

Standard length 24.9–57.0 mm., av. 40.6 (10). Proportional measurements: body depth 245–261, av. 255 (5); head length 268–291, av. 279 (5); head width 221–233, av. 226 (5); length anal fin 246–264, av. 257 (5); length pectoral fin 224–249, av. 238 (5); length pectoral spine 159–205, av. 183 (5); predorsal length 329–356, av. 345 (5); length dorsal spine 175–205, av. 189 (5). Meristic counts: total dorsal fin rays I, 7 (7); total anal fin rays 23–24, av. 23.6 (10); pectoral rays I, 8–I, 9, av. I, 8.8 (6); pelvic rays 8.0 (7).

Ameiurus natalis (LeSueur)
Yellow bullhead
(Table 8)

This species is common in the five Bladen County lakes, and is most abundant in Singletary where many were caught in gill nets. None of the body parts

TABLE 8

Proportional measurements and meristic counts in Ameiurus natalis *from the Bladen County lakes*

	JONES		SALTERS		SINGLETARY		WHITE		BLACK	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
	(7)		(3)		(11)		(24)		(6)	
Standard length (mm.)	14.7–222	73	51.2–274	176	36.0–113	57	22.0–183	62	26.9–168	88
	(6)		(3)		(4)		(10)		(5)	
Head length	265–311	276	293–301	298	271–292	281	254–280	265	276–303	290
	(6)		(3)		(4)		(10)		(5)	
Head width	224–260	242	238–291	257	224–241	230	221–231	225	230–253	240
	(6)		(3)		(11)		(24)		(6)	
Length anal fin	275–345	322	288–330	312	324–365	340	292–359	329	298–357	330
	(5)		(3)		(4)		(10)		(5)	
Length pectoral fin	169–196	181	163–181	171	166–197	185	157–197	183	167–234	203
	(5)		(3)		(4)		(9)		(5)	
Length pectoral spine	113–149	129	118–136	124	143–161	153	118–148	137	131–183	155
	(5)		(3)		(4)		(10)		(5)	
Length dorsal spine	110–136	127	108–133	122	139–160	147	119–141	133	112–165	132
	(6)		(3)		(11)		(24)		(6)	
Total anal rays	27–29	28.0	26–28	27.0	27–31	29.1	27–29	28.5	27–29	28.2

measured increases in size at a rate different from that of the body length. Hence the differences in Table 8 represent differences between the populations and not just chance variations depending on the average size of the individuals in the samples. The specimens from White Lake appear to have smaller heads both as to length and width than those from Salters Lake. The other series are intermediate. Length of the anal fin, as would be expected, is closely related to the total number of rays in the fin: in Singletary Lake the specimens have the longest anal fins and also the greatest number of fin rays, whereas the Salters Lake specimens have the shortest fins and the least number of rays. The Black Lake specimens appear to have distinctly longer pectoral fins than do the others. Since there is no detectable pattern to these differences it is likely that the yellow bullhead in these lakes is merely a greatly variable species.

Of the 116 specimens caught in gill nets the largest was a male from Salters Lake 321 mm. standard length, weighing 859 g. Only 2 other specimens, also males, were more than 300 mm. long. The rest ranged down to 136 mm. The mean lengths of these gill-net series ranged from 218 mm. (62) in Singletary to 264 mm. (6) in Jones.

Schilbeodes mollis (Hermann)
Tadpole madtom
(Table 9)

The tadpole madtom [= *S. gyrinus* (Mitchill, 1818); see Hubbs and Raney, 1944] occurs abundantly in all six lakes. It is readily distinguished from other madtoms of the region by the fact that the median fins are not edged with dark and the axial streak is quite distinct. The other characters, as listed in Table 9, confirm the identification and help define the limits of variation in the six populations.

There is considerable variation in the characters listed, but again, as in the case of *Ameiurus*, there is no distinct pattern. Moreover, even where there are rather large differences between any two series, the ranges of variation overlap so extensively that the differences might be entirely dependent on sampling. The two differences that may have some significance are the smaller number of anal rays in the series from White Lake and Waccamaw, and the smaller caudal ratio in the White Lake specimens. The latter character is quite apparent when the specimens are handled.

The only two proportional measurements that exhibit any change with increasing length of the fish are the head width and the length of the pectoral spine, the former decreasing slightly and the latter increasing slightly with increasing fish length.

Anguilla rostrata (LeSueur)
American eel

Four specimens of the American eel, 89–239 mm. total length, were collected in Lake Waccamaw, and 1 specimen 20–24 in. long was observed in White Lake at night. Quite likely eels are present in small numbers in the other lakes as well.

TABLE 9

Proportional measurements and meristic counts in Schilbeodes mollis *from the bay lakes*

	JONES		SALTERS		SINGLETARY		WHITE		BLACK		WACCAMAW	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
	(16)		(10)		(11)		(9)		(7)		(10)	
Standard length (mm.)	17.7–54.6	31.3	18.4–37.4	25.1	16.4–28.5	22.4	18.5–51.5	37.2	20.1–45.2	31.3	23.2–47.7	35.7
	(8)		(3)				(6)		(4)		(6)	
Predorsal length	350–377	364	358–405	383			324–369	354	360–391	377	313–380	355
	(8)		(3)				(6)		(4)		(6)	
Body depth	230–253	237	190–236	215			198–233	215	214–228	222	231–253	245
	(8)		(3)				(6)		(4)		(6)	
Head length	271–329	294	291–316	302			269–301	281	280–309	294	261–310	290
	(8)		(3)				(5)		(4)		(6)	
Head width	240–286	262	235–247	242			240–270	254	240–259	250	227–271	251
	(8)		(3)				(6)		(4)		(6)	
Length pectoral fin	206–268	240	235–263	246			192–231	214	225–252	237	212–235	221
	(8)		(3)				(6)		(4)		(6)	
Length pectoral spine	141–190	173	148–181	162			162–189	177	152–194	171	149–205	179
	(16)		(5)		(7)		(9)		(7)		(10)	
Caudal ratio[1]	1.2–1.5	1.36	1.3–1.5	1.38	1.3–1.5	1.40	1.1–1.4	1.24	1.1–1.5	1.30	1.3–1.5	1.40
	(16)		(10)		(7)		(9)		(7)		(10)	
Total anal rays	15–18	16.4	16–17	16.3	15–18	16.3	15–17	15.8	15–18	16.6	14–17	15.7

[1] Caudal ratio = $\frac{\text{distance from adipose notch to tip of caudal fin}}{\text{distance from adipose notch to posterior base of dorsal fin}}$

Fundulus waccamensis Hubbs and Raney
Waccamaw killifish

This species found only in Lake Waccamaw differs from its presumed parent species, *Fundulus diaphanus diaphanus*, in its more elongate form with a correspondingly greater number of scales and vertebrae. The specimens in the survey collection agreed in all respects with the description given by Hubbs and Raney (1946).

Standard length of 5 specimens 37.2–45.6, av. 40.9. Proportional measurements: body depth 171–188, av. 179; head length 280–293, av. 287; head depth 140–156, av. 146; head width 140–151, av. 145; predorsal length 561–570, av. 565; peduncle length 302–332, av. 318; peduncle depth 94–102, av. 99. Meristic counts: longitudinal scales 55–61, av. 59.2; transverse scales 18–19, av. 18.8; dorsal rays 14–16, av. 15.2; anal rays 12–13, av. 12.2; pectoral rays 17; pelvic rays 6.

Fundulus dispar lineolatus (Agassiz)
Southeastern starhead topminnow
(Tables 10, 11)

The above name has been assigned by Hubbs (Hubbs and Allen, 1943) to the species formerly known as *Fundulus notti* (Agassiz). This species was found in Jones, Salters, and White lakes.

The southeastern starhead was observed to reach a maximum size of 63.9 mm. in White Lake. The ventral contour is more strongly curved than the dorsal, which at times is almost straight. The profile is slightly concave over the eye. Coloration consists of a series of longitudinal stripes (typically 6) and a series of vertical bars (10 to 15) on each side. In small specimens, presumably immature, both series of markings are almost equally distinct. In mature males the vertical markings are more prominent, with the longitudinal stripes often completely absent. In females the stripes are more pronounced, although, except in the largest specimens, there are usually indications of the bars as well in varying degrees of distinctness. In addition to the stripes and bars there is a dark spot below the eye, and traces of up to five rows of spots on the anal fin and up to two rows of spots on the dorsal. These latter markings were found only in males.

Besides these color markings there are other differences between the sexes. In large males the origin of the anal fin is slightly closer to the snout than is the origin of the dorsal, whereas in females they are approximately the same distance away. Moreover, in males the dorsal and anal fins are distinctly larger than in females, and in males the anal is considerably larger than the dorsal.

There are some differences in coloration between the various populations. Females from White Lake always had 6 longitudinal stripes, whereas 3 specimens from Salters had traces of a seventh ventrad of the others. Two small specimens had only 5 stripes, but this may have been related to size. With respect to number of bars, the White Lake population seems to show a greater variability and at the same time a lower average number than the population from Salters. The number of bars is frequently not the same on the two sides of the same fish. Taking into consideration all specimens, both male and female, in which the bars

could be accurately counted, and counting the bars on each side separately, the number in White Lake ranged from 10 to 15 for 18 counts with a mean of 12.6, whereas in Salters the number ranged from 13 to 15 for 10 counts, with a mean of 13.7. One very small specimen from Jones had 9 faint bars on one side and 12 on the other. A mature male had 13 on each side.

The over-all coloration of the specimens from Jones and Salters is darker than of those from White Lake, and the membranes of the dorsal fins are often dusky rather than clear. This may be an environmental effect associated with the much darker dissolved color in Jones and Salters and almost complete absence of color in White Lake (Frey, 1949). Along this same line the subocular spot in

TABLE 10

Proportional measurements in Fundulus dispar lineolatus *from the Bladen County lakes*

	JONES		SALTERS		WHITE	
	Range	Mean	Range	Mean	Range	Mean
	(3)		(17)		(14)	
Standard length (mm.)	18.4–43.3	27.9	24.0–45.6	31.8	29.4–63.9	43.8
	(1)		(4)		(11)	
Body depth		224	216–221	218	191–241	220
	(1)		(4)		(11)	
Predorsal length		665	666–708	685	633–675	659
	(1)		(4)		(11)	
Head length		291	274–284	280	256–279	270
	(1)		(4)		(11)	
Peduncle length		243	243–256	251	241–273	253
	(1)		(4)		(11)	
Snout length		92	95–103	99	84–97	93
	(1)		(4)		(11)	
Eye diameter		97	84–86	85	72–85	79
	(1)		(4)		(11)	
Interorbital width		146	135–145	139	125–142	132
	(3)		(16)		(12)	
Lateral-line scales before dorsal fin	18–20	19.0	18–21	19.7	18–21	19.7

specimens from Jones and Salters was usually large and dark and sharply defined, whereas in White Lake specimens this spot was at times almost absent.

In proportional measurements (Table 10) the White Lake series differs from the others in having smaller heads and structures associated with the head—the eye and interorbital widths. These differences appear to be real and not associated with differences in size of individuals because in so far as could be determined from the meager series available, these three proportional measurements do not vary progressively with increasing size of fish. In addition the dorsal fin is somewhat farther forward in the White Lake specimens.

Meristically (Table 11) the White Lake specimens differ from the others by tending to have higher ray counts for the pectoral and pelvic fins, and a somewhat higher lateral-line count. All the Jones and Salters specimens had just 12

pectoral rays, whereas only 1 out of 14 White Lake specimens had a count this low. Comparison with University of Michigan material from N. C., S. C., and Ga. (Museum numbers 138561, 143213, 158085, and 88424) shows that the fin ray distribution in the White Lake specimens is more nearly typical than in the other two lakes. With respect to pelvic rays, however, the White lake material is

TABLE 11

Frequency of fin ray counts and scale counts in Fundulus dispar lineolatus *from the Bladen County lakes, with comparative material from N. C., S. C., and Ga. from the Univ. of Michigan Museum of Zoology*

	LAKE	MEAN	FIN RAYS										SCALES				
			5	6	7	8	9	10	11	12	13	14	34	35	36	37	38
Dorsal fin	Jones	8.0				2											
	Salters	7.9			2	14											
	White	8.2				11	3										
	N. C., S. C., Ga.	7.8		1	1	16											
Anal fin	Jones	10.0						2									
	Salters	10.1						15	1								
	White	10.2						11	3								
	N. C., S. C., Ga.	9.9					2	15	1								
Pectoral fin	Jones	12.0								2							
	Salters	12.0								16							
	White	13.0								1	12	1					
	N. C., S. C., Ga.	12.7								6	11	1					
Pelvic fin	Jones	6.0		2													
	Salters	5.9	1	15													
	White	6.6		6	8												
	N. C., S. C., Ga.	6.0		18													
Caudal fin	Jones	—															
	Salters	13.0								1	3	1					
	White	12.5								8	4	1					
	N. C., S. C., Ga.	12.4								11	5	1					
Lateral-line scales	Jones	34.3											2	1			
	Salters	36.0												5	6	5	
	White	36.5													8	5	1
	N. C., S. C., Ga.	34.6											10	5	3		

different. No other specimens examined had more than 6 pelvic rays, whereas more than half of the White Lake specimens had 7. In regard to lateral-line scales the series from both Salters and White lakes exhibit higher average counts than the comparative river series in the Museum. This difference, however, may well be another example of a common type of difference between fluviatile and lacustrine populations of the same species.

Considering all characters, the White Lake population is probably different from those in Jones and Salters, but more material is needed to define the magnitude of these differences.

Gambusia affinis holbrooki Girard
Eastern gambusia

This small, widely distributed species was found most abundantly in White Lake, with lesser numbers collected in Singletary and Waccamaw. It was not collected or seen in the other three lakes in Bladen County. The latter fact is surprising, because the mosquitofish occurs abundantly throughout this entire region. Possibly the general lack of aquatic plants in these lakes and high predation rates have kept the species from becoming established.

Menidia extensa Hubbs and Raney
Waccamaw glassminnow

This very interesting species was described by Hubbs and Raney (1946). More recently Gosline (1948) has shown that it is undoubtedly a fresh-water derivative of *M. beryllina*. It is not now known to occur at any place besides Lake Waccamaw.

Morone americana (Gmelin)
White perch

This species, likewise occurring only in Waccamaw, is the most important species for angling in the lake. The fish apparently move about in large schools offshore. Fishermen can often catch the legal limit of these fish in a very short time. The population is typical of the species.

Standard length 123–158 mm., av. 131 (8). Proportional measurements: body depth 302–331, av. 314 (8); head length 351–382, av. 368 (8); eye diameter 82–89; av. 86 (8). Meristic counts: longitudinal scales 48–49, av. 48.4 (8); scales above lateral line 6–8, av. 7.3 (8); scales below lateral line 11–12, av. 11.5 (8); spines first dorsal 9 (8); spines second dorsal 1 (8); soft rays second dorsal 11–12, av. 11.9 (8); anal spines and soft rays III, 9 (7).

Micropterus salmoides salmoides (Lacépède)
Northern largemouth bass
(Table 12)

The largemouth bass is abundant only in White Lake and Waccamaw, although it also occurs sparingly in Jones and Salters. Only one specimen was taken from each of the latter two lakes. Unfortunately the specimen from Jones Lake was not preserved, and the one from Salters is quite badly bloated and distorted.

The specimens from White and Waccamaw agree fully with the description of *M. salmoides salmoides* by Hubbs and Bailey (1940) and Bailey and Hubbs (1949). Table 12 summarizes the body proportions and meristic counts for the samples from these two lakes. The fingerlings and yearlings have the typical lateral row of dark blotches, more or less confluent. Larger specimens are more

uniformly dark in color with a rather faint dark speckling on an otherwise lighter lower half. There is no tendency, however, for the spots to line up in rows as in *M. punctulatus*.

As is true for many species in these lakes, various proportional measurements change with increasing size of the individuals. In this species the body depth

TABLE 12

Proportional measurements and meristic counts in Micropterus salmoides salmoides *from the bay lakes*

	WHITE		WACCAMAW		SALTERS
	Range	Mean	Range	Mean	1 specimen
	(15)		(8)		
Standard length (mm.)	67.3–245	118	63.0–201	93	276
	(15)		(6)		
Body depth	244–301	273	250–309	276	307
	(15)		(6)		
Head length	338–382	360	344–369	358	344
	(15)		(6)		
Eye diameter	52–85	70	61–80	73	50
	(15)		(7)		
Scales: longitudinal	61–71	65.9	62–67	65.3	70, 66[1]
	(13)		(8)		
Scales: above lateral line	7–9	7.7	7–8	7.8	8
	(15)		(8)		
Scales: below lateral line	13–16	14.5	14–16	14.6	16
	(15)		(3)		
Scales: circum peduncle	26–28	26.9	26–28	27.0	28
	(15)		(8)		
Scales: cheek rows	9–12	10.5	10–11	10.3	10
	(15)		(8)		
Spines: 1st dorsal	8–9	8.9	8–10	9.0	9
	(15)		(8)		
Spines: 2nd dorsal	0–1	0.9	1–2	1.1	1
	(15)		(8)		
Soft rays, 2nd dorsal	12–14	12.8	12–13	12.9	13
	(15)		(8)		
Anal spines	2–3	2.9	3	3.0	3
	(15)		(8)		
Anal soft rays	11–12	11.3	10–12	11.0	11
	(15)		(8)		
Pectoral rays	14–15	14.5	14–15	14.8	14

[1] The larger count on the left side includes several small intercalated scales.

increased from 267 for specimens 50–74 mm. standard length to 291 for specimens longer than 200 mm. Over this same size range eye diameter progressively decreased from 79 to 54. Head length remained approximately constant, or showed just a slight decrease over the size range represented.

The single specimen from Salters Lake is typical *salmoides salmoides* except for the presence of well developed rows of scales on the interradial membranes

TABLE 13

Proportional measurements and meristic counts in Chaenobryttus coronarius *from the bay lakes*

	JONES		SALTERS		SINGLETARY		WHITE		BLACK		WACCAMAW	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
	(2)		(3)		(15)		(11)		(5)		(4)	
Standard length (mm.)	183–195	189	62.7–112	83	78.3–192	126	39.5–158	95	35.0–98.6	54	110–133	125
	(2)		(3)		(15)		(11)		(3)		(4)	
Body depth	441–442	442	387–424	402	376–456	413	376–466	416	390–395	393	422–451	434
	(2)		(3)		(15)		(11)		(3)		(4)	
Head length	399–409	404	414–431	424	396–443	413	382–429	399	418–428	424	392–424	415
	(2)		(3)		(15)		(11)		(3)		(4)	
Eye diameter	78–81	80	110–118	114	81–125	104	80–127	99	114–136	125	89–99	92
	(2)		(3)		(15)		(11)		(3)		(4)	
Scales: longitudinal	43	43.0	43–45	43.7	42–47	44.6	42–48	45.1	43–44	43.7	44–48	45.8
	(2)		(3)		(15)		(11)		(3)		(4)	
Scales above lateral line	8	8.0	7	7.0	7–8	7.6	6–8	6.9	7	7.0	6–8	7.3
	(2)		(3)		(15)		(11)		(3)		(4)	
Scales below lateral line	13–14	13.5	12	12.0	12–13	12.4	12–14	13.2	12–13	12.7	12–13	12.8
	(2)		(3)		(15)		(11)		(5)		(4)	
Dorsal spines	10	10.0	9–10	9.7	9–10	9.8	9–10	9.9	9–10	9.8	9–10	9.8
	(2)		(3)		(15)		(11)		(5)		(4)	
Dorsal soft rays	10	10.0	10–11	10.3	9–11	10.1	10–11	10.4	10–11	10.2	10	10.0
	(2)		(3)		(15)		(11)		(5)		(4)	
Anal soft rays[1]	9–10	9.5	9–10	9.3	9–10	9.5	9–10	9.5	9–10	9.8	9–10	9.5

[1] All specimens had 3 anal spines.

of the dorsal and anal fins. In some places these rows of scales extend out more than 2 cm. onto the membranes. This specimen, kindly examined by R. M. Bailey, has been deposited in the University of Michigan Museum.

Chaenobryttus coronarius (Bartram)
Warmouth
(Table 13)

The warmouth is the only centrarchid besides *Enneacanthus* that occurs in all the lakes. It was most abundant in Singletary Lake, where large numbers of fish of the year were taken, indicating good reproduction. In the other lakes most of the specimens captured were mature fish, the largest measuring 195 mm. standard length.

As in other species for which fairly extensive series were studied, the body proportions of the warmouth change with increasing length. The body depth markedly increases and the eye diameter decreases. The head length seems to show a slight decrease with increasing size, although the trend is by no means obvious. There are no certain differences in body proportions between the various populations.

In this species there is an interesting correlation between the number of scales in the lateral line and the transparency of the water. Even in the small samples examined, fish from the clearest lakes (Waccamaw and White) had higher lateral-line counts than those from the darkest lakes (Jones, Salters, and Black), with Singletary being intermediate. A similar correlation was found in *Perca*, q. v.

Lepomis
(Table 14)

Species of the genus *Lepomis* were collected only in Lake Waccamaw and in White and Singletary lakes. The species evidently occur in Waccamaw naturally but they have probably been planted in the Bladen County lakes. There are State records of bluegills having been stocked in White Lake in 1942, and there almost certainly have been unrecorded plantings of this and other species in the past.

An interesting feature of the records is that 600 bluegill fingerlings were introduced into Jones Lake in 1943 and 10,000 fingerlings into Singletary in 1942 as well as 165 six-inch fish in 1944. The only evidence of survival of these fish was one partly eaten centrarchid, identified as a bluegill, recovered from a gill net in Singletary. No young bluegills were caught in either lake. Evidently some conditions in the dark-colored lakes of Bladen County have prevented the successful establishment of these fishes. These might be in part the scarcity of vegetation and the high predation pressure resulting from generally low productivity.

Even in White Lake conditions are not very favorable for *Lepomis*. No young of *L. auritus* were seen or collected, even though quite a few redds were observed (Hueske, 1948) and only a few *L. macrochirus* of any size were taken in the collections. By way of contrast, in Waccamaw large numbers of fry and fingerlings

of all centrarchids present, except *Enneacanthus*, were easily collected. There is no apparent differentiation of these several populations in the two lakes.

TABLE 14

Proportional measurements and meristic counts in species of Lepomis *from White Lake and Waccamaw*

	L. PUNCTATUS (Waccamaw—2)		L. GIBBOSUS (Waccamaw—4)		L. AURITUS (Waccamaw—6) (White—2)		L. MACROCHIRUS (Waccamaw—20) (White—3)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Standard length	(2)		(4)		(8)		(23)	
(mm.)	49.3–104	73	81.5–114	94	72.0–183	123	52.1–181	106
	(2)		(4)		(7)		(23)	
Body depth	471	471	451–510	481	421–465	445	411–560	506
	(2)		(4)		(7)		(23)	
Head length	349–371	360	348–359	355	361–388	377	324–401	353
	(2)		(4)		(7)		(23)	
Eye diameter	89–122	106	85–102	94	80–107	94	77–129	96
			(4)				(15)	
Length pectoral fin			330–361	348			289–388	338
	(2)		(4)		(8)		(23)	
Scales: longitudinal	41	41.0	43–47	44.5	44–54	49.0	45–50	47.3
Scales: above lat-	(2)		(4)		(8)		(23)	
eral line	7	7.0	7	7.0	7–9	8.0	7–8	7.7
Scales: below lat-	(2)		(3)		(8)		(23)	
eral line	13–14	13.5	13–14	13.3	14–16	15.1	14–16	14.8
	(2)		(3)		(5)		(4)	
Scale rows on cheeks	6–7	6.5	4–5	4.7	8–9	8.2	5	5.0
	(2)		(4)		(8)		(23)	
Dorsal spines	10–11	10.5	10	10.0	9–10	9.9	10	10.0
	(2)		(4)		(8)		(23)	
Dorsal soft rays	10–11	10.5	11–12	11.3	11–12	11.3	10–13[1]	12.0
	(2)		(4)		(8)		(23)	
Anal soft rays[2]	10–11	10.5	10	10.0	9–10	9.8	10–12	11.3
			(4)		(8)		(23)	
Pectoral rays			13–14	13.3	13–15	14.1	13–14	13.7

[1] The only specimen with 10 soft rays in the dorsal fin had an injured fin. The range of variation in the normal specimens in the collection is 11–13.

[2] All specimens had 3 anal spines.

Lepomis punctatus punctatus (Valenciennes)
Eastern spotted sunfish

Hubbs and Raney (1946) listed an unknown species of *Lepomis* from Waccamaw known locally as "grass perch." Hueske (1948) reported that the fishermen claim there is a hybrid sunfish in the lake, although no specimens turned up in the collection. Instead there were two specimens of the eastern spotted sunfish (kindly identified by R. M. Bailey), which in many respects is intermediate between *auritus* and *gibbosus* and could very easily be mistaken for a hybrid. It has a stiff opercle and rounded pectoral fins. The gill rakers are intermediate in

length between *gibbosus* and *macrochirus*. The maxilla extends beyond the anterior edge of the eye. The breast scales are considerably larger than in *auritus*, numbering only about 20 rows.

Fowler (1945) lists numerous literature records as well as collection records for this species from South Carolina, Georgia, and Florida. Previously (1912) he had listed the species from Southport, North Carolina, the first record for the State. Apparently the spotted sunfish is not too common in the North Carolina Coastal Plain. Other common names for the species listed by Fowler are chinquapin perch, black perch, and black bream.

Lepomis gibbosus (Linnaeus)
Pumpkinseed

Occurring only in Lake Waccamaw the pumpkinseed is relatively unimportant in the creels although the young may help to support the more important bass and crappies and other predators in the lake. None of the 79 specimens taken during the survey was very large, the biggest measuring 124 mm. standard length.

Lepomis auritus (Linneaus)
Yellowbelly sunfish

Larger specimens of this species (up to 189 mm. standard length) were taken in White Lake than in Waccamaw. A White Lake specimen with a deformed maxilla that prevented the jaws from closing completely had exceptionally long ear flaps, extending back to the base of the sixth dorsal spine.

Lepomis macrochirus purpurescens Cope
Southeastern bluegill

The bluegill in Lake Waccamaw is abundant and is important not only in the creels of the fishermen but also in providing forage fry and fingerlings for the piscivorous species. Hueske (1948) regarded the bluegill as the most important fish in the lake in this respect. In White Lake the species is not nearly so abundant. Only a few specimens were collected by the survey crew, and the species is poorly represented in the creels. Large specimens from both lakes tend to lose their vertical bars, as is typical of *purpurescens*. The Waccamaw specimens demonstrate a decided increase in body depth and a less marked decrease in eye diameter with increasing length.

Enneacanthus gloriosus (Holbrook)
Bluespotted sunfish
(Tables 15, 16)

One of the two centrarchids present in all the lakes is the little bluespotted sunfish. It is considerably more abundant relative to other species in the lakes of Bladen County than in Waccamaw, and it is more abundant in the colored lakes than in White Lake.

These populations are uniform in that the opercular spot is about one-half the

diameter of the eye, a little more or less. All the scales of the lateral line are perforated except for perhaps the last scale on the caudal peduncle. The posterior edge of the maxilla extends to or just beyond the anterior edge of the orbit. All have pigmentation along the second and third pelvic rays, giving the appearance of a dark stripe just inside the lighter leading edge. The spine of the pelvic fin extends to or beyond the anus in all specimens except some from White and Waccamaw.

The populations in White Lake and Waccamaw are distinct in certain characteristics (Table 15), which may well be ecotypic. The White Lake specimens have a distinctly shallower (more elongate) body than those from the other lakes, and the specimens from White Lake and Waccamaw, particularly the latter, have longer heads than those from the other lakes. Moreover, the pelvic fins are

TABLE 15

Proportional measurements in Enneacanthus gloriosus *from the bay lakes*

LAKE	STANDARD LENGTH (MM.)		BODY DEPTH		HEAD LENGTH		EYE DIAMETER	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
	(5)		(5)		(5)		(5)	
Jones	34.9–56.0	45.6	425–467	446	389–399	395	121–140	132
	(2)		(2)		(2)		(2)	
Salters	54.7–54.9	54.8	440–456	448	375–379	377	124	124
	(29)		(15)		(15)		(15)	
Singletary	32.8–61.2	44.6	435–500	465	378–413	396	115–131	123
	(5)		(5)		(5)		(5)	
White	34.3–48.3	37.9	384–418	404	378–425	402	126–134	129
	(9)		(9)		(9)		(9)	
Black	47.3–69.2	62.1	439–503	475	369–397	380	111–127	116
	(2)		(2)		(2)		(2)	
Waccamaw	35.4–39.2	37.3	437–449	443	411–429	420	127–130	129

shorter both in White Lake and Waccamaw. In these populations the pelvic spine just barely reaches the vent, and the longest soft ray extends only to the base of the first, or at the most second, anal soft ray. In the other populations the pelvic spine extends past the anus, and the tip of the fin reaches the base of the third anal soft ray. The specimens from Black Lake have a greater number of scales above and below the lateral line than those from the other lakes (Table 16). This difference may well be an apparent one, related to the larger average size of the Black Lake specimens, which would make it less likely that any small scales had been missed in the counts. In spite of these differences the populations are on the whole quite uniform and exhibit little significant differentiation.

The bluespotted sunfish is quite uniform in its proportional measurements over the size range represented. There is only a relatively slight and uncertain tendency for the body depth to increase, the head length to decrease, and the eye to decrease in size with increasing length. The numbers of specimens studied were not sufficient to permit detection of differences between the various populations in these characters.

TABLE 16

Meristic counts in Enneacanthus gloriosus *from the bay lakes*

	NO.	JONES	SALTERS	SINGLETARY	WHITE	BLACK	WACCAMAW	TOTAL
Lateral-line scales	30		1	3	2		1	7
	31		1	6	1	2	1	11
	32	1		6	1	4		12
	33	2		3	1	1		7
	34	1		1		2		4
	Mean	32.6	30.5	31.6	31.2	32.3	30.5	31.8
Scales: above lateral line	4			6	3		2	11
	5	4	2	13	1	4		24
	6				1	5		6
	Mean	5.0	5.0	4.7	4.6	5.6	4.0	4.9
Scales: below lateral line	10	4	2	14	4	2	2	28
	11			4	1	6		11
	12			1		1		2
	Mean	10.0	10.0	10.3	10.2	10.9	10.0	10.4
Dorsal spines	8	1		3	2			6
	9	2	2	26	3	9	2	44
	10	1						1
	Mean	9.0	9.0	8.9	8.6	9.0	9.0	8.9
Dorsal soft rays	10	1	1		1	1		4
	11	2		24	3	8	1	38
	12	1	1	5	1		1	9
	Mean	11.0	11.0	11.2	11.0	10.9	11.5	11.1
Anal spines	2			1				1
	3	4	2	28	5	9	2	50
	Mean	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Anal soft rays	10	3	1	14	4	6	1	29
	11	1	1	15	1	3	1	22
	Mean	10.2	10.5	10.5	10.2	10.3	10.5	10.4

Centrarchus macropterus (Lacépède)

Flier

(Table 17)

The flier was found in all the lakes except Waccamaw. It was most abundant in Singletary Lake. Fewer specimens were taken in Jones and Black, and only a

single specimen each was collected in Salters and White. The specimens from the dark-colored lakes are quite uniform in their characters, and accordingly, because of their small frequencies, they have been lumped together in Table 17. The single specimen from White Lake, however, differs so markedly in a number of respects that it deserves special treatment. Its lateral-line count is much smaller that in other specimens, and the number of scales below the lateral line and the

TABLE 17

Proportional measurements and meristic counts in Centrarchus macropterus *from the Bladen County lakes, with comparative material from unpublished data of R. M. Bailey*

	COLORED BLADEN LAKES[1]		WHITE LAKE	COMPARATIVE MATERIAL			
				S. C.		Total	
	Range	Mean	1 specimen	Range	Mean	Range	Mean
Standard length (mm.)	(12) 37.1–92.1	64	131				
Body depth	(9) 428–485	463	492				
Head length	(9) 373–420	384	361				
Eye diameter	(9) 116–129	121	87				
Scales: longitudinal	(9) 42–43	42.6	36	(66) 37–44	40.0	(165) 36–44	39.7
Scales: above lateral line	(9) 6–7	6.7	7				
Scales: below lateral line	(9) 13–14	13.4	12				
Dorsal spines	(12) 12	12.0	12	(98) 11–13	11.9	(200) 11–14	12.3
Dorsal soft rays	(12) 13–14	13.8	13	(96) 12–15	13.4	(196) 12–15	13.2
Anal spines	(12) 7–8	7.1	8	(98) 7–8	7.6	(201) 7–9	7.7
Anal soft rays	(12) 15–16	15.3	14	(97) 13–16	15.1	(200) 13–17	14.9

[1] Frequencies: Jones—1; Salters—1; Singletary—9; Black—1.

number of anal soft rays are likewise smaller. Other counts agree with the ranges of variation in the specimens from the brown-water lakes.

In Table 17 there are shown for comparison some previously unpublished scale and ray counts for the species from a manuscript by R. M. Bailey. The White Lake specimen is extreme in its count of lateral-line scales. Not one of 66 South Carolina specimens had a count this low, and only 3 out of a total of 165 specimens over the entire range of the species in the U. S. had such a low count. The number of anal soft rays, although low compared with the other Bladen County material, falls well within the range of the species as a whole. It would be desirable to have more specimens from White Lake to ascertain the extent of variation in these characters.

Pomoxis nigro-maculatus (LeSueur)
Black crappie

The black crappie in Waccamaw, the only lake of this group in which the species occurs, is one of the two most important fishes to the ordinary angler. It reaches an observed maximum size of 274 mm. standard length and a weight of 574 g. The population in the lake agrees closely with the description of the species.

Standard length 38.5–60.8 mm., av. 47.1 (10). Proportional measurements: body depth 342–366, av. 349 (5); head length 367–390, av. 381 (5); eye diameter 99–107, av. 102 (5). Meristic counts: longitudinal scales 45–48, av. 46.5 (2); scales above lateral line 6–7, av. 6.7 (3); scales below lateral line 14 (2); dorsal spines 7–8, av. 7.1 (10); dorsal soft rays 14–15, av. 14.7 (10); anal spines 5–6; av. 5.9 (10); anal soft rays 17–19, av. 17.6 (10).

Perca flavescens (Mitchill)
Yellow perch
(Tables 18–20)

The yellow perch is common in all six lakes investigated. In White Lake and Waccamaw large schools of small perch numbering in the hundreds or even thousands of individuals are quite frequently seen. The species grows to a large size in these lakes, attaining a maximum observed size of 315 mm. standard length and a weight of 652 g. These large specimens which live mainly offshore are caught by trolling. They are known locally as "redfin perch" or "raccoon perch."

In proportional measurements there was little detectable difference between the populations, although there was a suggestion of a lesser body depth and snaller head length in the White Lake specimens, and of smaller eyes in the White Lake and Waccamaw series. Such conclusions based on the present small samples, however, are tenuous because of the marked differences in rate of increase of the various body parts. Table 18 demonstrates that there is a decided increase in body depth with increasing size and a concomitant decrease in eye diameter. Head length may possibly decrease somewhat with increasing size. As a result of this differential growth young perch present the typical "hunger form" of stunted populations, although in the present instance the phenomenon is probably ontogenetic rather than nutritional.

As to meristic characters there are no pronounced differences between the several populations in the number of rays in the dorsal or anal fins (Table 19), but there are differences in the number of scales in longitudinal and transverse series. Either coincidentally or causally the number of pored scales in the lateral line (or the number of lateral-line scales, since all of them were pored) shows a direct correlation with the transparency of the lakes (Table 20). Six specimens from White Lake, in fact, had one or two pored scales on the scaly covering of the caudal fin; these are not included in the data in the table.

In number of scales above and below the lateral line, the populations in White Lake and Waccamaw tend to be more variable than those in the other lakes.

TABLE 18

Change in proportional measurements of Perca flavescens *from the bay lakes with increasing size*

STANDARD LENGTH (MM.)	NO.	BODY DEPTH		HEAD LENGTH		EYE DIAMETER	
		Range	Mean	Range	Mean	Range	Mean
25–49	2	228–229	229	330–336	333	88–92	90
50–74	11	211–250	232	302–356	323	78–94	84
75–99	29	219–257	240	301–360	330	67–93	81
100–124	7	234–257	244	300–349	314	61–77	70
125–149	4	244–265	258	292–351	322	62–71	67
150–199	2	247–273	260	291–317	304	57–58	58
200–249	3	262–273	267	301–310	305	50–54	52
250–325	4	254–297	284	305–338	320	45–50	48

TABLE 19

Variation in dorsal and anal ray counts in Perca flavescens *from the bay lakes*

		NO. OF RAYS	JONES	SALTERS	SINGLETARY	WHITE	BLACK	WACCAMAW	TOTAL
First dorsal fin		12		1[1]					
		13			3	1			4
		14	9	4	11	13	4	10	51
		15				2	2	4	8
		Mean	14.0	14.0	13.8	14.1	14.3	14.3	14.1
Second dorsal fin	Spines	1	1	1	1	2	2	3	10
		2	8	4	13	13	4	11	53
		3				1			1
		Mean	1.9	1.8	1.9	1.9	1.7	1.8	1.9
	Soft rays	13			1	1		2	4
		14	2	5	7	9	2	10	35
		15	5		6	6	2	1	20
		16	2				2	1	5
		Mean	15.0	14.0	14.4	14.3	15.0	14.1	14.4
Anal fin[2]		7	2	2	2	2		6	14
		8	6	3	12	13	6	8	48
		9	1			1			2
		Mean	7.9	7.6	7.9	7.9	8.0	7.6	7.8

[1] Spinous dorsal deformed in this specimen; at least one ray missing.

[2] All specimens had 2 anal spines.

The mean number of scales below the lateral line in these two lakes considered as a single population is significantly greater ($t = 5.8$ for 59 d. f.) than that of

TABLE 20

Scale counts in Perca flavescens *from the bay lakes*

	NO. OF SCALES	JONES	SALTERS	SINGLETARY	WHITE	BLACK	WACCAMAW
Pored scales in lateral line	50		1				
	51	1					
	52					2	
	53						
	54						
	55	3	1		1	1	
	56	1	1	1	1		1
	57	1	1	2		1	1
	58	1	1	2	1	1	1
	59	1		2	2	1	4
	60			1	2		1
	61	1		2	4		2
	62			1	2		2
	63				1		1
	64						1
	65						
	66				1		
	67						
	68						
	69						
	70				1		
	Mean	56.3	55.2	58.9	60.9	55.5	60.0
Av. secchi disc in ft.[1]		2.4	1.8	2.5	11+	1.8	4.4
Av. color in ppm.[1]		297	299	168	<10	182	160
Scales below lateral line	12		1	1		1	
	13	4	1	6	2	4	1
	14	3	3	3	4	1	3
	15	2		1	8		6
	16				2		1
	17						2
	18						1
	Mean	13.8	13.4	13.4	14.6	13.0	15.2
Scales above lateral line	6			1	4	1	
	7	6	2	8	3	2	7
	8	3	3	2	5	2	7
	9				2	1	
	10				2		
	Mean	7.3	7.6	7.1	7.7	7.5	7.5

[1] Data from Frey, 1949.

the pooled populations of the other four lakes. Since there are no marked or consistent differences in the standard length:depth ratio in the lakes, the only

conclusion is that the scales of the White Lake and Waccamaw perch below the lateral line are either relatively narrower or overlap more extensively than those of the other lakes.

Boleosoma perlongum Hubbs and Raney
Waccamaw darter

This species of *Boleosoma* is differentiated along much the same lines as the other two endemic fishes in Lake Waccamaw in being relatively more elongate than related species of the surrounding region (Hubbs and Raney, 1946). The scale counts and vertebral counts are higher, and there are differences in the number of head pores. The 6 specimens examined agree with the original description of the species.

Hololepis thermophilus Hubbs and Cannon

This diminutive species of darter is abundant in all the bay lakes investigated except Black Lake, where no specimens were collected and where consequently the species is probably not established. Detailed study of all the specimens available (Bailey and Frey, 1951) showed that the populations in the dark-colored lakes of Bladen County differ quite markedly from those of White and Waccamaw, particularly in their lesser number of lateral line pores. Accordingly two subspecies were recognized: typical *thermophilus* for the populations in lakes White and Waccamaw, and a new subspecies for the populations in Jones, Salters, and Singletary.

The darters in general are notable for their phenotypic (and presumably genotypic) plasticity. Considering the age of these lakes it is not surprising to find that *Hololepis thermophilus* exhibits differentiation of taxonomic significance. In some respects the trend of differentiation parallels that in *Perca*, which is considered to be ecotypic, but in other respects it runs directly counter to the expected trends. Hence, in spite of certain anomalies in distribution, the populations in the three dark-colored lakes are thought to be a valid genetic phenotype rather than an ecotypic variation.

Uncertain records

In the preliminary report on these fish faunas Hueske listed a number of records that are neither represented by specimens in the collection nor mentioned in the field notebooks. All of these should be regarded as doubtful, or at least provisional, records until they can be authenticated by specimens.

1. *Amia calva*. A specimen 280 mm. standard length in the collection was labelled "Jones Lake, July 30, 1947, Lot 48, Fish 256." Lot 48 is not listed in the field book, but Lot 47 from White Lake on this same date has a bowfin of identical size listed as Fish 250. Specimen 256 in the records is a perch. Because of the confusion in the records the specimen has been assigned to White Lake on circumstantial grounds. Hueske, however, regards the Jones Lake record as *bona fide*.

2. *Ameiurus natalis*. In the field notes for Lake Waccamaw the large ameiurids collected are variously listed as catfish, bullheads, or yellow bullheads. Specimens listed as catfish or bullheads were found to be *Ictalurus catus*. Unfortunately

none of the 12 specimens recorded as yellow bullheads is in the collection. Since there should be no confusion in the field between these markedly dissimilar species, there is a good possibility that *Ameiurus* is present in Waccamaw.

3. *Gambusia affinis* was listed by Hueske as occurring in all the lakes, although there were no records or specimens for Jones, Salters, or Black lakes. The author is inclined to regard this species as absent from these 3 localities.

4. *Aphredoderus sayanus*. A single specimen of the pirateperch was supposedly collected in Black Lake, but again there is no record in the field book and no specimen in the collection.

Fish Faunas of the Bladen County Lakes

It is likely that the Bladen County lakes have always been strongly acidic, as they are at present. The surrounding soils are primarily sandy, with little or no lime in the surficial formations. Because of the acidity, low calcium content, and brown color in most of them and the consequent low level of productivity and curtailment of growth of higher aquatic plants, the fish faunas of these acid lakes are much poorer in species than the fauna of Lake Waccamaw, and have probably always been poorer.

The number of native species is small and is quite constant from one lake to another. All the Bladen County lakes have a core of the following species:

1 pike (*Esox americanus*)
1 sucker (*Erimyzon sucetta*)
1 bullhead (*Ameiurus natalis*)
1 madtom (*Schilbeodes mollis*)
3 sunfish (*Chaenobryttus coronarius*, *Enneacanthus gloriosus*, *Centrarchus macropterus*)
yellow perch (*Perca flavescens*)
1 darter (*Hololepis thermophilus*)

Except for the absence of *Hololepis*, the above list represents the complete fish fauna known from Black Lake. In the other lakes of Bladen County various other species have either gained access to the lakes naturally or have been introduced by man.

Fundulus and *Micropterus* show a distribution correlated with drainage pattern and probably determined by this pattern. These two fishes occur in the 3 lakes—Jones, Salters, and White—that drain into the Cape Fear River near Elizabethtown (Fig. 1) but not in Singletary and Black lakes in the other arm of the drainage system.

Gambusia and *Esox niger* show peculiar patterns of distribution that may be more closely correlated with various chemical and physical or biotic conditions in the lakes than with the drainage. *Gambusia* is known for certain only from the two most transparent and likewise most productive lakes of the series—White and Singletary. Both these lakes have considerably more littoral vegetation than the other three. *Esox niger* occurs in Singletary Lake as well as Jones and White lakes. These 3 lakes are considerably more productive than Salters and Black in terms of total numbers of individuals. In the latter two lakes the chain pickerel,

although undoubtedly having access to the waters, evidently cannot maintain itself in competition with the smaller predators such as the redfin pickerel and warmouth.

Certainly the occurrence of *Amia* and *Anguilla* in White Lake is natural, and one might expect small populations of these predators to be present in the colored lakes as well, even though no specimens were taken during the survey.

A striking feature of the faunas is the complete lack of cyprinids, except for *Notropis chalybaeus* in Singletary. It is the author's opinion that this species is a very recent addition to the lake. About 1935 the Resettlement Administration dug an outlet channel 160 yards long through the sand rim of Singletary Lake and constructed a spillway dam at the outer end. This channel provides conditions not found at any other place in the Bladen County lakes. The bottom is almost completely covered with heavy growths of *Sphagnum*, and along the shore are patches of *Sacciolepis*, *Xyris*, and *Nymphaea*. Evidently the conditions in this outlet channel are particularly favorable to *Notropis*, because out of a total of 391 specimens collected, all but 2 were taken either in the channel or along the adjacent lake shore. The population is centered in the channel, spreading out from there into the lake where the fish undoubtedly enter the food chains leading to the top predators. *Notropis chalybaeus*, which is a common species of the region, could have gained access to the lake from the effluent directly or become established through the release of bait fishes by fishermen.

It is not likely, as previously stated, that any of the species of *Lepomis* are native to the Bladen County lakes. The apparent failure of a number of recorded plantings of bluegills indicates that conditions in these rather unproductive lakes are not generally favorable for the species, and the small numbers of *macrochirus* and *auritus* from White Lake indicate that even when established the species are not too successful.

The fish faunas of these five lakes are topheavy in predacious species. Hueske (1948) found that 52 per cent of the total number of organisms in the stomachs of 22 largemouth bass from White Lake were darters (*Hololepis thermophilus*). The other small species in the lakes, *Schilbeodes mollis*, is abundant and is probably taken by the larger predators, particularly the largemouth. Other species that are undoubtedly important as forage for the piscivorous species are small perch and small suckers. In addition, the young of *Enneacanthus*, the most abundant sunfish in these lakes, are available. The populations of *Fundulus* and *Gambusia*, although apparently quite small, may aid in supporting the large predators. Of the rough fishes, *Erimyzon* is most abundant in Singletary Lake, and *Ameiurus* is most abundant in White and Singletary.

FISH FAUNA OF LAKE WACCAMAW

The fish fauna of Waccamaw is much more diversified than those of the other lakes studied. It is likewise more extensive than indicated by Hubbs and Raney (1946); they listed 17 species as probably occurring in the lake, whereas in the present report there are specimen records of 25 species.

The water of the lake is in direct contact along the north shore with a calcareous

Miocene formation, from which it apparently obtains enough calcium to raise the pH of the inflowing swamp water to neutrality and thereby increase the transparency and the general level of productivity. Production of all types of aquatic organisms is noticeably greater than in the Bladen County lakes. Rooted aquatic plants are more diversified and much more abundant, with maiden cane forming vast beds extending offshore for more than 1000 feet in a number of places. Large emergences of Ephemeridae occur in spring. Molluscs, both vivalves and univalves, are abundant. The fish populations are large and support an extensive sport fishery. Finally, the outlet stream from Waccamaw is large and itself supports a local pole and line fishery; it provides a ready access to the lake for all species in the upper part of the Waccamaw River. Hence, Lake Waccamaw has been more readily accessible from a large stream than the Bladen County lakes, and because of its size, chemical conditions, and generally greater productivity, including a diversity of littoral habitats, it has facilitated the establishment of a greater number of species.

The core of native species present in the Bladen County lakes is also present in Waccamaw, except for *Esox niger* and *Centrarchus macropterus*, which were not recorded during the survey. *Ameiurus* is not known with certainty from the lake, but *Ictalurus* is common.

The major additions to the fauna have been a number of centrarchids and cyprinids, another large predator (*Lepisosteus*), the gizzard shad, and several more small species that can serve as food for the larger predacious species (Table 1). There is no reason for considering any of these species as introductions. Although largemouth bass and bluegills, and more recently crappies, have been stocked in the lake, the purpose of these actions was to bolster existing populations rather than to introduce new species.

Hubbs and Raney (1946), not knowing the complete composition of the fish fauna, were perhaps inclined to place too great emphasis on predation pressure as a selection factor bringing about the attenuated body form of the three endemic species described by them. Besides these three small species and *Notropis* there are also another darter (*Hololepis*) which is apparently more abundant than *Boleosoma*, the golden shiner, the gizzard shad, and the tadpole madtom, to say nothing of the numerous young of the bluegills, pumpkinseeds, white perch, and yellow perch. Certainly at the present time there are adequate populations of small fish to support the predators, and there does not seem to be any good basis for believing that predation pressure was substantially greater before development of the present sport fishery, which is mainly for non-piscivorous species.

DISCUSSION

Pollen analysis of the sediments of Singletary Lake reveals an oak-hickory maximum in post-glacial time, following a period containing more mesophytic species such as hemlock and beech, and followed by a pine-oak dominance (Frey, 1951). The period of the oak-hickory maximum is interpreted as being considerably drier than the present. The middle organic layer, known to be more

than 20,000 years old, likewise was probably formed during a dry period, as indicated by large percentages of grasses, composites, and shrubs in the pollen spectra. If at either or both of these times the lakes were isolated long enough geographically for any of the species of fish in them to develop reproductive isolation from the fluviatile populations of the region, then, providing the lacustrine populations have been able to survive to the present, some degree of morphological differentiation would be expected. Because in many species of organisms there has been little detectable differentiation since the last period of glaciation, it is likely that any effective period of isolation was contemporaneous with the middle organic layer in Singletary Lake or older.

Presumably Lake Waccamaw was isolated in such a manner because it has 3 species of fish (*Fundulus waccamensis*, *Boleosoma perlongum*, and *Menidia extensa*) that so far as known occur only in the lake and are likely valid endemic species. The other 22 species in the lake exhibit no marked deviation from populations of these species elsewhere.

If the 5 lakes in Bladen County have enjoyed approximately the same ontogenetic history as Waccamaw (leaving chemical factors out of consideration), and there is no reason to believe that they have not, then one would expect to find endemics in them as well. Actually, however, the differentiation is much less extensive in these lakes than in Waccamaw. *Fundulus* from White Lake is different in a number of respects from the species as it occurs in two nearby lakes and from the extra-lacustrine populations examined. *Erimyzon* in the Bladen County lakes appears to differ from populations elsewhere in having supernumerary breeding tubercles as a regular character. The greatest degree of differentiation occurs in *Hololepis thermophilus*, although this too is on a subspecies rather than a species level. The general lack of differentiation in these lakes is believed to be primarily a result of their rather poor fish faunas, composed of quite stabile species. The only "plastic" species present is the darter, and, as mentioned above, this does exhibit a certain amount of differentiation interpreted as genetic. (See Bailey and Frey, 1951.)

When the differences between the populations of the various species in the several lakes are compared, certain patterns become apparent that are more closely correlated with ecological conditions than with drainage systems. Thus, the populations of the dark-colored lakes in Bladen County tend to form one group, and those in White Lake and Waccamaw another, even though the two latter lakes are in entirely different drainages. In *Perca flavescens* the White and Waccamaw specimens have a larger number of lateral-line pores and scales and a greater number of scales below the lateral line. In *Hololepis thermophilus* the populations in White and Waccamaw have more lateral-line pores, but about the same number of or slightly fewer total lateral-line scales. In *Enneacanthus gloriosus* the relatively more attenuated body in the White Lake fish and the shorter pelvic fins in the specimens from White and Waccamaw have already been pointed out. In *Chaenobryttus coronarius*, as in the yellow perch, the White Lake and Waccamaw fish have a greater number of lateral-line scales. The single specimen of *Centrarchus macropterus* from White Lake tends to be somewhat

atypical in number of lateral-line scales and anal soft rays, and at least is markedly different from the other Bladen County specimens. The *Erimyzon* specimens from White Lake seem to have fewer scales in transverse series than do those from the dark-colored lakes. Finally, the differentiation of *Fundulus* in White Lake as compared with the populations in Jones and Salters might be recalled. In other words, except for the 3 endemic species in Waccamaw and the possible genetic differentiation of 3 other species in the Bladen County lakes, differences between the populations follow ecological lines rather than drainage patterns. It is believed that such differences are in fact largely ecotypic. Inasmuch as the 3 endemic species in Waccamaw are extreme examples of the type of modification discussed above, the question is raised to what extent they, too, are ecotypic rather than genetic species.

The prevailing tendency is for the populations from lakes White and Waccamaw to have greater meristic counts and smaller body parts such as fins and head than the corresponding populations in the colored lakes of Bladen County. According to the discussion of Martin (1949), such differences suggest a slower rate of development in White and Waccamaw. These two lakes have less color and a greater transparency than the other lakes (Table 20), but probably of greater significance they have the greatest average depths of water of all the lakes—7.6 ft. in Waccamaw, and 7.5 ft. in White Lake (Frey, 1949). The other lakes range from 7.0 ft. in Singletary to 5.3 ft. in Black Lake.

These differences appear rather slight, but coupled with the greater light absorbing capacity of the colored waters they may help bring about a more rapid warming of the water in spring in the colored lakes of Bladen County than in White and Waccamaw. Substantiating data are not available at present. If true, however, it would provide an explanation for the resemblance of the White and Waccamaw populations through a slower rate of metabolism during development of eggs spawned in early spring.

The other type of variation that occurred almost constantly in the species results from the differential growth of the body parts. The body depth usually increased and eye diameter decreased with increasing size. The head either decreased in relative length or remained approximately constant. These ontogenetic changes made it difficult to detect significant differences in body proportions between the various populations.

As a final remark it should be mentioned that the Bladen County fishes are living in natural waters with a pH between 4.3 and 4.9. It was formerly believed, and statements to that effect still are being added to the literature, that fishes are unable to tolerate such low pH levels, but of course the present populations are living in these lakes with no ill effect.

SUMMARY

1. A total of 32 different species and subspecies of fishes are reported from 6 natural Coastal Plain lakes of North Carolina. Lake Waccamaw has 25 species, White Lake 17, and Black only 8. The other three have 11–13 species. Except for Waccamaw the lakes have a pH less than 5.

2. A detailed study of the body proportions and meristic characters of the populations did not reveal any endemic species in the Bladen County lakes. Possible genetic differentiation was confined to *Fundulus*, *Erimyzon*, and *Hololepis*.

3. In general population characteristics the dark-colored lakes of Bladen County (Jones, Salters, Singletary, and Black) resembled one another, and White and Waccamaw resembled each other, even though the latter two lakes are in entirely different watersheds. Because the differences observed seemed to be associated with ecologic conditions, they are interpreted as being primarily ecotypic rather than genetic.

4. Many of the species with adequate numbers over a wide size range exhibited certain definite trends in change of body proportions with increasing size: the body depth increased, the eye decreased, and the length of head either remained approximately the same or else decreased somewhat.

5. Because no differences could be found in proportional measurements, meristic characters, coloration, or distribution of breeding tubercles between the population of *Notropis* in Lake Waccamaw and two populations of *Notropis petersoni* from outside the lake, the designation *Notropis waccamanus* Fowler for the lacustrine population has been reduced to a synonym of *Notropis petersoni* Fowler.

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STUDIES ON PHYTIN

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Introduction

During the past decade increasing attention has been directed to the availability in human nutrition of certain dietary essentials in the foods that contain them. The fact that the gross total content of some constituents, as indicated by *in vitro* methods of laboratory analysis, does not necessarily give an accurate indication of the amount which can be utilized after ingestion of the food in question has become increasingly apparent. A prominent case in point is that of phosphorus. The daily adult human requirement of this element is about one gram. This amount may be supplied by a variety of foods among which milk holds a prominent place. Also, judged by gross analysis, most of the grains which make up our common cereals should be effective sources of phosphorus and it has been somewhat disappointing to find that a large share of this phosphorus in wheat, corn, and oats, as well as in other cereals, is incapable of being digested or absorbed and is therefore unavailable, at least to the human organism. The unavailable form of phosphorus in such cereals is known as "phytin." It is a mixed calcium and magnesium salt of the hexaphosphoric acid ester of the hexahydric alcohol inositol.

The fundamental obstacles to biological utilization of this compound are first, its insolubility and second, the lack of any enzyme normally present in the human gastro-intestinal tract which can effect its hydrolysis, either as the calcium or magnesium salts or as the much more soluble sodium salt. As a result, there is lost by fecal elimination the phosphorus, the calcium and magnesium, and the inositol, the latter now having assumed a place among the minor members of the vitamin B complex. Although there is at present little or no evidence of inositol deficiency in human nutrition, any enzymic digestive process which would hydrolyze phytin would contribute to the supply of physiologically available inositol. The object of the work reported in this paper was to study some biological agents which might be effective in rendering available the various dietary constituents of this compound.

Historical

Posternak (1903a) isolated for the first time an organic phosphorus compound from plant grains. He obtained a yield of about 2% of the dried grain. The resulting substance was found to be a mixture of the calcium and magnesium salts of a phospho-organic compound with a phosphorus content of about 22%. A few months later Posternak (1903b) described a method for the preparation of the free acid. Shortly afterwards there appeared a publication by Gilbert and

Lippmann (1904) recommending the administration of phytin to human patients as a rich source of assimilable phosphorus. Their recommendation was based on the high content of phosphorus in phytin (about seven times that of lecithin and thirty-one times that of casein). It took into account no question at all as to the ability of the organism to utilize this phosphorus.

From this time to the present there have appeared more than forty papers dealing with the chemistry and the nutritional aspects of this compound. The consensus of opinion throughout has been that in human nutrition, phytin, either in cereal foods or as a pharmaceutical preparation, is of little or no value. Furthermore, the presence of either free phytic acid or of the sodium salt has been shown by certain investigators to have an actual rachitogenic effect. By combining in the small intestine with dietary calcium from other sources it renders the calcium insoluble and thus prevents its absorption. On diets, marginal with respect to calcium, this effect has been quite thoroughly demonstrated (Bruce and Callow; 1934, Harrison and Mellanby, 1939; McCance and Widdowson, 1942; Henry and Kon, 1945; Hoff-Jorgensen *et al.*, 1946). The present nutritional status of the phytin problem is well reviewed by Mollgaard (1946).

Various structures for phytin have been suggested in years past but it is now fairly well agreed that phytic acid is the hexaphosphoric acid ester of inositol. In the absence of proof to the contrary, it seems most logical to assume the presence of six orthophosphoric acid groups, each esterified with the six alcoholic groups of inositol and leaving twelve replaceable hydrogen atoms per molecule. In no case has any salt been prepared in which more than twelve equivalents of cations are present. Our investigation of the titration curve of this compound, described below, confirms this structure.

Much conflicting evidence exists in the literature concerning the presence of phytin-splitting enzymes in the gastro-intestinal tract and in other parts of the body. In some cases the presence of such enzymes in the gastro-intestinal tract was judged by the proportion of phytin, administered by mouth, which finally appeared in the feces. For example, McCance and Widdowson (1935) fed phytin to several human subjects and found from 20 to 60% of the dose to be excreted unchanged in the feces. It should be noted, in view of our own results described below, that hydrolysis of even 40 to 80% of the administered phytin in the intestine might take place but at so low a point as to render impossible any absorption of the products.

Another reason for the variable results obtained by different investigators may be found in the choice of experimental animals. Lowe and Steenbock (1936) demonstrated the partial hydrolysis of phytin in the intestines of rats while Patwardhan (1937) reported the isolation of a phytin-splitting enzyme from rat's intestines. Lowe and Steenbock suggested that consideration should be given to the role of intestinal flora in this hydrolysis. An elaborate study of the absorption of calcium, magnesium, and phosphorus and of the influence of phytin on this absorption was reported by McCance and Widdowson (1942a). Their conclusions are in general agreement with those we have outlined above.

In view of these results, we have felt that more consideration should be given

to the role of microorganisms in the hydrolysis of phytin. We have desired particularly to test for enzymic activity some of the microorganisms representing the flora of water, soil, and the intestinal tract.

EXPERIMENTAL

The product used as the starting point for this work was a commercial phytin purchased from the Ciba Pharmaceutical Products, Inc., of Summit, N. J. It was labeled as the "calcium magnesium salt of inositol hexaphosphoric acid," and showed the following analysis:

	per cent
Water (drying at 110°C)	6.00
On the dry basis:	
Calcium	9.60
Magnesium	0.74
Total phosphorus	20.00
Inorganic phosphorus	2.70
Phytin phosphorus	17.30

The above figures represented no satisfactory stoichiometrical relation between the metals and the phytin phosphorus and indicated the product to be a mixture of phytates, probably with less than complete substitution of the replaceable hydrogen atoms of phytic acid. To produce a phytate of more definite composition the barium salt was prepared according to the method described by Patwardhan (1937). In this method, an acidified solution of the commercial phytin was treated with barium hydroxide in the presence of ethyl alcohol and with a final adjustment of the pH to 8.4. Three such precipitations of the barium salt gave a product which was dried to constant weight over P_2O_5 at room temperature and which gave the following analysis:

	per cent
Barium	46.7
Inorganic phosphorus	0.4
Phytin phosphorus	9.2

Barium was determined in the usual way as barium sulfate. Inorganic phosphate was determined colorimetrically by the Gomori (1942) procedure, to which phytin phosphorus does not respond. Total phosphorus, determined by the same procedure on a sulfuric acid digest of the barium salt, corrected for the inorganic phosphate, gave, by difference, the phytin phosphorus.

On the assumption that the 0.4% inorganic phosphorus was present as tribarium phosphate (at pH 8.4), the above barium figure may be correspondingly corrected by 2.7%, leaving 44.0% barium. This, as compared with the 9.2% of phytin phosphorus, gives atomic ratios for the two of 0.32 and 0.30 respectively, thus implying equimolar amounts of barium and phosphorus. The hexabarium phosphate, on further drying to constant weight at 110°C., lost 2.3% of its weight. This calculates to a loss of two molecules of water for the compound as dried at room temperature but it also implies the presence of a large amount of combined water which is not even removed by drying at 110°C. The compound,

dried to constant weight at 110°C., contained 45.0% barium and 9.42% phosphorus. It is suggestive to note that hexabarium phytate with addition of 24 moles of combined water has a theoretical composition of 43.3% barium and 9.76% phosphorus. Assuming the possibility of contamination with a small amount of some other barium salt, this implies a stable combination of four moles of water with each esterified phosphoric group in the phytate molecule. Subsequent examination confirmed the presence of small amounts of barium chloride of such magnitude as to account for the slightly higher barium and lower phosphorus percentages indicated above.

Sodium phytate was prepared from the above described barium salt by dissolving one-gram portions in 60 ml. 0.1 N. hydrochloric acid and adding sulfuric

TABLE I

Titration of Phytic Acid with Sodium Hydroxide

MILLILITERS 0.1 NORMAL SODIUM HYDROXIDE	MILLIMOLES NaOH PER MILLIMOLE OF PHYTIC ACID	pH
0	0	1.80
1	2	1.90
2	4	2.40
2.5	5	3.00
3.0	6	5.10
3.5	7	6.00
4.0	8	7.08
4.5	9	8.30
5.0	10	9.40
5.5	11	10.90
6.5	13	11.20
7.0	14	11.50
8.0	16	11.60
10.0	20	11.70
15.0	30	11.70

acid equivalent to the barium content of the solution. The acid solution resulting from removal of the barium sulfate was then neutralized with sodium hydroxide to obtain those solutions used for the hydrolysis experiments. To obtain free phytic acid the equivalent amount of sulfuric acid was added directly to one-gram portions of the barium salt. The barium sulfate was filtered and the resulting solution made up to 100 ml., of which 10 ml. portions were taken for determination of the titration curve of phytic acid as described below.

Titration curve of phytic acid. The 10.0 ml. portions of phytic acid, each containing 0.0502 millimoles phytic acid, were titrated with 0.100 N. sodium hydroxide using a Leeds and Northrup glass electrode for the determination of pH. The results are recorded in Table I.

The accepted structure of phytic acid implies the existence of twelve replaceable hydrogen atoms per molecule of which six, the first of each pair, should dissociate at a fairly low pH, somewhat analogous to the first hydrogen

of phosphoric acid. The second of each pair might be expected to have a much higher pK value. The data in Table I confirm this conclusion, although a titration curve including twelve constants can obviously not be expected to produce twelve sharp individual inflections. As in the case of the titration curves reported for various proteins, such a multiplicity of constants results in a fusion of these inflections. The first six moles of alkali added per mole of phytic acid gave a somewhat more vertical curve lying between pH 2 and 5. This part of the curve is succeeded by a comparatively flat portion which soon begins the gradual rise to about pH 11. This latter branch of the curve includes the second set of six inflections. These appear to be somewhat more widely spaced than were the first six, resulting in a more gradual slope of the curve. After pH 11 the remainder of the curve is merely one of dilution of the added alkali. We may conclude, therefore, that the first hydrogen atom of each pair dissociates at pK values from two to about five, whereas the second of each pair lies between pK 6 and pK 11 with much wider spacing between individual members of the second group than of the first.

Enzymic hydrolysis of phytin. Stock cultures of 24 different microorganisms were secured through the courtesy of the Department of Bacteriology. These represented the most common flora usually found in the intestinal tract, in water, and in soil. All were subcultured in meat extract broth, a culture medium composed of meat extract, proteose-peptone, sodium chloride, and water. The more nutrient meat infusion broth media could not be used because of their high content of inorganic phosphate. The use of such a relatively simple culture medium limited the number of microorganisms that could be tested since a great proportion of them require not only meat infusion media but also the addition of small amounts of protein. In preparing a substrate for the hydrolysis experiments two different methods were developed after many trials.

1. The broth on which the microorganism had grown was diluted with sterile broth to a uniform turbidity corresponding to a concentration of one billion organisms per ml. using the McFarland nephelometric method (Kolmer and Boerner, 1941). The resulting solution was divided into two parts. One of these was mixed with an equal volume of the solution of sodium phytate, prepared as described above. The other served as a control to be used after incubation. Both suspensions were incubated at 37°C. for 24 hours. At the end of this period they were cooled to room temperature, centrifuged at 3000 r.p.m. in an angle centrifuge for 20 minutes and the clear supernatant liquid used for the phosphate determinations. From this supernatant liquid, 0.5 ml. samples were taken for the phosphate determination while from the broth control 0.25 ml. samples were mixed with the same volume of the sodium phytate solution immediately before developing the color in both solutions.

The concentration of total and of inorganic phosphate was previously determined in the broth alone and in the mixture of broth and phytate. In evaluating the results the difference between the inorganic phosphate content of the two solutions after 24 hours incubation was taken as due to hydrolysis of the sodium phytate by an enzyme produced by the microorganism under examination.

2. The second method was similar but with the only difference that after the turbidity of the suspension of microorganisms had been standardized it was centrifuged until a perfectly clear solution was obtained. The supernatant liquid was divided into two parts; one of them was heated in boiling water for half an hour to destroy any possible enzyme and then cooled to room temperature. Equal amounts of each one of the solutions and of sodium phytate were mixed in two test tubes and after incubation at 37°C. phosphate determinations were made, using 0.5 ml. of each solution.

This method had to be limited to an incubation period of 18 hours after which growth of the microorganisms began to be apparent, due apparently to the impossibility of sterilizing the solution completely by centrifugation and heating as described. One difficulty encountered with both methods was that when the molybdate-sulphuric acid reagent was added a turbidity developed. However, after the volume had been adjusted to 15 ml. the precipitate could be easily filtered, giving a perfectly clear blue solution. The precipitate was due to some substance present in the broth since the sodium phytate alone failed to give any precipitate on addition of the molybdate reagent. Moreover, it was apparently not due to a protein, since the addition of trichloracetic acid failed to precipitate any proteins and did not prevent the formation of the precipitate with the reagents. It was thought that the use of the broth could probably be eliminated by growing the organisms in agar and using a saline suspension of the growth. In this case, however, very little hydrolysis of the phytate resulted with any organism.

The results of the hydrolysis experiments using the first method already described with a period of 24 hours incubation are given in Table II. The results expressed in this table were all obtained from stock cultures after only one passage through broth. It should be stated that these results were by no means consistent; in some instances a microorganism would produce a certain degree of hydrolysis after repeated subcultures in broth. In other cases the degree of hydrolysis would be less after several passages. It is clear, however, from the average results in Table II that several microorganisms produce a certain limited degree of hydrolysis of the sodium phytate.

We desired to test the effect of a prolonged incubation period and also the effect of adding a fresh culture to the substrate. For this purpose a standardized suspension of *E. coli* was mixed with an equal volume of the solution of sodium phytate and incubated for six days, at the end of which an equal volume of fresh broth was added and the mixture further incubated for four more days. Aliquots were taken each day for inorganic phosphate determinations. The results are given in Table III. It is evident from this table that the degree of hydrolysis increased up to the third day, after which it remained constant. The addition of new broth slightly increased the degree of hydrolysis of the sodium phytate.

It is noteworthy that the microorganisms which are capable of producing a certain degree of hydrolysis of the phytin molecule belong to entirely different groups. Thus, while *E. coli* is a normal inhabitant of the intestinal tract *A. aerogenes* is rarely found there, but is normally present in water. The shigellas

TABLE II

Hydrolysis of sodium phytate after 24 hours incubation at 37°C

Substrate in all cases contained 0.6 mg. P per 1 ml.

MICROORGANISM	SOURCE	REMARKS	% HYDROLYSIS
Escherichia coli	Intestine	Normal	12.6
Aerobacter aerogenes	Intestine	Normal	18.1
Alkaligenes faecalis	Intestine	Normal	0
Proteus vulgaris	Intestine	Normal	0
Streptococcus faecalis	Intestine	Path.	0
Salmonella typhosa	Intestine	Path.	0
Salmonella paratyphi	Intestine	Path. typhoid	0
Salmonella schottmuelleri	Intestine	Path. typhoid	0
Salmonella aertrycke	Intestine	Path.—food poisoning	0
Salmonella enteritidis	Intestinal	Path.—diarrhea	0
Salmonella suipestifer	Intestinal	Path.—hog cholera	0
Salmonella cholerasuis	Intestinal	Path.—gastroenteritis	0
Shigella dysenteriae	Intestinal	Path.—dysentery	2.3
Shigella paradysenteriae	Intestinal	Path.—dysentery	1.1
Shigella sonnei	Intestinal	Path.—dysentery	13.0
Pseudomonas aeruginosa	Intestine and air	Path.—infections	0
Brucella abortus	Milk	Path.—undulant fever	0
Vibrio metchnikovi	Intestine	Path.—cholera in chickens	4.1
Staphylococcus aureus	Skin		0
Pseudomonas fluorescens	Water		1.3
Staphylococcus citreus	Air		2.5
Serratia marcescens	Air and intestine	No clinical significance	0
Sarcina lutea	Air		0
Bacillus subtilis	Air		0

TABLE III

Hydrolysis of sodium phytate by E. coli after different periods incubation

TIME INTERVAL IN DAYS	MGM. TOTAL P PER 1 ML. SUBSTRATE	MGM. P SET FREE	% HYDROLYSIS
1	0.60	0.070	11.6
2	0.60	0.082	13.6
3	0.60	0.096	16.0
4	0.60	0.096	16.0
5	0.60	0.096	16.0
6	0.60	0.096	16.0
(new broth added)			
7	0.37	0.070	18.0
8	0.37	0.075	20.0
9	0.37	0.075	20.0
10	0.37	0.075	20.0

are all pathogenic but the degree of hydrolysis does not parallel their pathogenicity, *S. dysenteriae* being the most pathogenic to man. All of these already

mentioned are, however, Gram negative, *E. coli* and *A. aerogenes* being lactose fermenters and the shigellas non-lactose fermenters. *Pseudomonas fluorescens*, like *A. aerogenes*, is normally found in water and soil and *Staph. citreus* is also a saprophyte. The latter is the only one of all organisms showing any activity which has a coccus shape. *Vibrio metchnikovi* is pathogenic to fowl.

It is possible that many of those microorganisms not producing any enzyme might do so by repeated culture in a medium containing phytin. The phosphorus requirements of a microorganism may have some relation to its ability to obtain it from other sources than inorganic phosphate. However, the possible hydrolysis of phytin in the intestinal tract due to microbic action is apparently not practicable since this hydrolysis would take place in the large intestine, where absorption is negligible.

SUMMARY

From commercial phytin a recrystallized hexabarium phytate was prepared and analyzed. From the barium salt there was prepared both the sodium salt and free phytic acid.

The titration curve of free phytic acid with alkali was determined by means of the glass electrode and the approximate positions of each of the two main groups of six ionizable hydrogen atoms on the pK scale were determined.

A solution of sodium phytate was prepared from the barium salt and used as a substrate to test for phytase activity in a number of microorganisms from air, soil, and the intestinal tract. From 24 microorganisms tested, 8 showed some ability to hydrolyze sodium phytate. The others gave negative results.

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THE SYNTHESIS OF CHOLINE (METHYL-C^{14}) CHLORIDE CONTAINING METHYL-C^{14}

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The preparation of choline (methyl-C^{14}) chloride was undertaken at the request of Dr. Camillo Artom of the Bowman Gray School of Medicine, Winston-Salem, N. C. The compound had not at that time been reported in the literature, although Du Vigneaud and Verly (1) reported the *in vivo* preparation of it after this work was completed.

An attempt was made to prepare the compound following the directions used by Du Vigneaud (2) in preparing choline chloride containing deuterium; the method consists of condensing methyl iodide with ethanolamine in alkaline solution, followed by precipitation of choline as the reineckate and decomposition of the reineckate; yields using this method proved to be unsatisfactory. The method finally developed is shown in the accompanying equations; β-dimethylaminoethanol (I) was condensed with labeled methyl iodide (II) in benzene solution at room temperature; choline iodide (III) precipitated in substantially quantitative yield. Choline iodide was changed into choline chloride by shaking with an aqueous suspension of freshly precipitated silver chloride; the yield was over 95%.

$$\underset{\text{I}}{(CH_3)_2NCH_2CH_2OH} + \underset{\text{II}}{C^{14}H_3I} \xrightarrow{\text{benzene}} \underset{\text{III}}{[(CH_3)_2\overset{\displaystyle C^{14}H_3}{\overset{|}{N}}CH_2CH_2OH]^+ I^-}$$

Anhydrous choline chloride could be obtained by evaporation *in vacuo*; this was done for analytical purposes, but the main portion of the labeled choline chloride was kept in solution made 0.1 N with hydrochloric acid.

Several runs using inactive materials indicated the over-all yield to be better than 95% based on methyl iodide; the choline chloride and choline iodide were both isolated and analyzed for nitrogen. Analysis for β-dimethylaminoethanol by the method of Artom and Crowder (3) showed it to be absent from the choline chloride solution.

The active material was prepared using one millicurie of methyl iodide supplied by Tracerlab, Inc.; analysis of the choline chloride showed it to contain an activity of one millicurie per millimole.

Experimental

Twenty runs were made with inactive materials to find the optimum conditions; the final run made with the "hot" methyl iodide is described below.

The tube containing one millimole of methyl-C^{14} iodide (fitted with a break-off

seal) obtained from Tracerlab was sealed to the vacuum line and the line evacuated. A dry-ice acetone bath was placed around the methyl iodide; it was noted that the liquid did not solidify, as pure methyl iodide does at that temperature. The seal was broken, and the methyl iodide allowed to distill into a conically shaped reaction tube containing 20 ml. of benzene, the tube and contents being cooled with liquid air while the tube containing the methyl iodide was warmed to room temperature. When the transfer was complete the reaction tube was allowed to come to room temperature and 1.5 millimoles (0.133 g.) of β-dimethylaminoethanol (b. p. 133–135°) was added; a drying tube was attached and after shaking to insure mixing the solution was set aside for 48 hours. It was noted that a cloudiness developed within five minutes, and a copious precipitate formed within an hour.

After 48 hours the solution was cooled to 5°C. and the benzene removed with the aid of a filter stick; the reaction tube was placed on the vacuum line and the last traces of benzene removed *in vacuo*. The trace of choline iodide adhering to the filter stick was rinsed into the reaction tube with 5 ml. of water. The carefully washed silver chloride formed from 2 g. of silver nitrate, was added to the tube, and the tube shaken on the shaker overnight. The aqueous solution was filtered from the yellow silver iodide with a clean filter stick; the precipitate was washed with several small amounts of water, each being added to the original filtrate until 10 ml. had been collected. A small quantity (0.083) ml. of conc. hydrochloric acid was added to the solution. After stirring, an aliquot was removed for carbon 14 analysis; this was done by evaporating the sample to dryness, burning to carbon dioxide, and measuring the activity in an ionization chamber on a Vibrating Reed Electrometer.

With the method above using inactive materials, yields of 95–98% of choline chloride based on methyl iodide were realized. A sample of inactive choline iodide was analyzed. Calcd. for $C_5H_{14}ONI$: N, 6.06. Found: N, 5.94, 6.05. A sample of inactive choline chloride was also analyzed. Calcd. for $C_5H_{14}ONCl$; N, 10.0. Found: N, 9.95, 10.1.

Acknowledgment: The aid of Dr. Paul H. Cheek in some of the analytical work and of Dr. R. L. McKee in discussing the problem and supplying the dimethylaminoethanol is gratefully acknowledged. This work was done under a contract between the Atomic Energy Commission and the Bowman Gray School of Medicine, Winston-Salem, N. C.; Contract No. AT-(40–1)-288, Title II; Principal Investigator, Dr. Camillo Artom.

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HOW DIFFICULT PARTURITION IN CERTAIN VIVIPAROUS SHARKS AND RAYS IS OVERCOME

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Eighteen Figures

Introduction

This paper deals with difficult parturition in certain viviparous Elasmobranchs, the lowest group of the present-day fishes—far down toward the bottom of the vertebrate sub-phylum of the chordata. But such difficult parturition is not unknown among the mammals, the highest of the vertebrates.

It can be assumed that the student, who comes to take a course in comparative anatomy of vertebrates, knows that the unborn kitten or lamb (mammals) is carried in the womb (uterus) of the mother until it is ready to be born. He also possibly knows that the embryo has a "navel string" or umbilical cord with its blood vessels and that the outer end of this is expanded into an organ called a placenta ("afterbirth"), which is attached to the wall of the womb and from which it gets and transfers foodstuff to the young mammal. And he also judges that this is true of the highest mammals—the genus Homo.

But when he comes to dissect a pregnant viviparous shark, great will be his astonishment to find, in this lowest of the fishes, reproductive organs which at least are recognizable forerunners of what are found in the mammals (including man) at the very top of the vertebrate group. He finds on each side of the shark's abdomen an oviduct extending the length of the cavity. In front is an opening into which the large egg slips when set free from the ovary. About midway of the tube, it passes into the shell gland and acquires a thin shell. Further back the egg comes to rest in the much enlarged uterus or womb. Here the thin shell is discarded. The growth of the embryo takes place at the expense of the food material in the egg. In some viviparous sharks the emptied yolk sac and its vitelline vessels become affixed to the inner wall of the maternal uterus and serve as a functional placenta. In some of the rays the young are nourished by a secretion from the outgrowths from the inner wall of the uteri called villi or trophonemata—as will be seen later in certain figures.

To the present writer, this parallelism in reproductive organs in animals at the opposite poles of the vertebrate sub-phylum is a most amazing thing in zoology.

However, this paper has to do with difficult parturition in certain viviparous sharks and rays. Such, indeed, is not unknown in the highest vertebrates, the mammals. Among domestic animals, two-headed calves and other monstrosities cause difficult parturition. In the highest of the mammal group, the genus Homo, multiple births—triplets, the rare quadruplets and the still rarer quintuplets—cause many difficulties. But far more difficult are the births of such abnormalities as the so-called "Siamese twins."

These abnormals in mammals, however much difficulty they cause the mother in parturition, are very infrequent. In the elasmobranchs under study, the young which cause the difficulties in parturition are normal foetal sharks and rays. Furthermore, some of them are possessed of normal organs, which, unless they are provided with protective devices, would at parturition bring great disturbance and possible death to the mothers. It is a study of these particular devices for easing parturition that will now be undertaken.

I have for years been filing away notes from articles in which are found brief accounts incidental to this interesting but little studied subject of difficult parturition in certain viviparous Elasmobranchs and how it is provided for. No attempt has ever been made to bring together the widely scattered data and now it seems worth-while to organize these accounts into an orderly presentation for the record.

The Elasmobranchii, or strap-gilled (non-operculate) fishes, include the Sharks and Rays. In the viviparous rays, as well as in certain viviparous sharks, because of the general structure of the foetuses and particularly of certain of their organs, parturition would seem to be impossible. To show how Nature has facilitated such difficult births is the purpose of this article.

PARTURITION IN CERTAIN VIVIPAROUS SHARKS—SELACHII

Sharks are large-bodied Elasmobranchs, constructed on a fore and aft axis. They are large forward where the feeding apparatus is located, and are materially smaller in the region of the cloaca which delimits the body and tail sections. This relatively smaller size of the abdominal cavity at the rear, together with peculiarities in the structure of some embryos, makes for difficult parturition in certain forms.

IN THE HAMMERHEAD SHARKS

These grotesque fishes are distinguished from all other sharks by the remarkable shape of the head, which in the eye region of each side of the head is produced into a conspicuous lobe. These lobes are supported internally by cartilaginous outgrowths from the skull. Each lobe bears an eye at its outer extremity.

As seen in the fishes or their portrayals, the ordinary hammerhead has long T-shaped lateral projections of the head with the eyes at the ends. On the other hand, the shovelhead has the lateral processes bluntly rounded and the head is somewhat kidney-shaped. For these processes in the young (not foetuses, however) of the two forms, see Fig. 1 copied from T. W. Bridge in the Cambridge Natural History (Fish Volume), 1904, p. 450. These are the antipodes of the hammerhead tribe. From these drawings it is clear that parturition in the bonnethead will be fairly easy—but how can it be effected in the exaggerated hammerhead (*S. zygaena*)? There must be some temporary adaptation to make the birth of the young possible; the explanation was long ago found and will be considered in its place.

Two different hammerheads have been found by dissection to be viviparous, and all others are presumed to be live-bearers. The data for the two noted—

Sphyrna tiburo and *Zygaena blochii*—will now be presented. The first is not a pronounced hammerhead but the latter is—as the figures show.

Sphyrna tiburo—the Shovelhead

The shovelhead or bonnethead has very short and rounded hammers—in fact its head, as Fig. 1 shows, is somewhat kidney-shaped. All other hammerheads

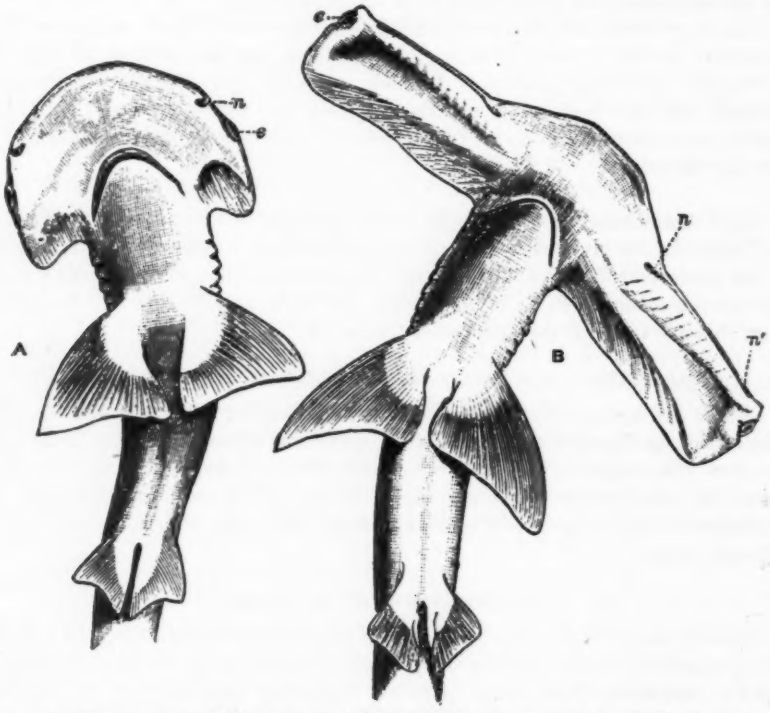

FIG. 1. The antipodes of the hammerhead tribe: (A) the rounded head of a Bonnet- or Shovelhead Shark (*Sphyrna tiburo*) and (B) the elongate hammers of a pronounced hammerhead (*Sphyrna zygaena*)—n, nostril, e, eye. (After Bridge, 1904.)

are just that and *tiburo*, because of its shovel-like head, is sharply marked off from its fellows.

A good deal is known about viviparity in the shovelhead and it will be of interest and value to present these accounts first and chronologically.

I.—From Brasil—1836. The first to see and certainly to figure a foetus of *S. tiburo* is Leuckart (1836, pp. 22–24, pl. 3). His little specimen came from a pregnant female "2 Fuss und 15 bis 16 Zoll" long taken on the coast of Brasil. This foetus he figures in both dorsal and ventral aspects. Since a better figure is at hand of the foetus in dorsal view, that showing Leuckart's little fish from the

under surface is reproduced herein as Fig. 2. Note the pronounced kidney-shaped head with the rounded hammers, the ventral mouth, the external gills, the long yolk stalk with the thick outgrowth of "appendicula." This is the oldest known figure of a hammerhead embryo.

II.—From Beaufort, North Carolina, U.S.A.—1902 and 1911. In 1902, the present writer, at a seashore laboratory for the first time, had the good fortune to help dissect a pregnant female bonnethead shark brought in from Cape Lookout. Being innocent of any knowledge of viviparity in sharks, I hardly realized this good fortune. However, in 1911, I assisted in the capture and dissection of a second pregnant female, and later published a preliminary note

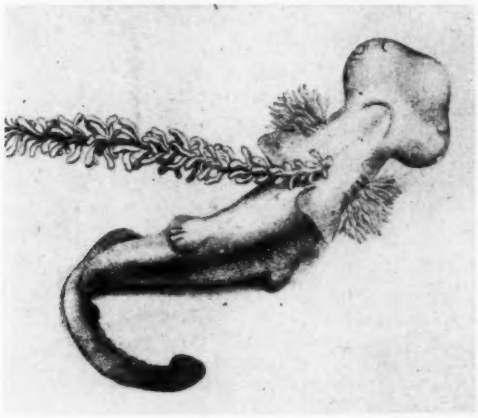

FIG. 2. The earliest figure of an intra-uterine foetus of the bonnethead shark, *Sphyrna tiburo*. Note the rounded hammers, the profuse external gills, and the yolk cord with appendicula. Not being essential to the study, the outer end of cord with the yolk sac has been omitted. (After Leuckart, 1836).

on the intrauterine embryos and in 1912 (pp. 143–4), a somewhat fuller description of these.

The mother was 50 inches over all, the foetuses about 50 mm. and corresponded generally to Leuckart's figures. My notes say that the external wall of the uterus was firm, tough, and muscular. The lining mucous membrane was very wrinkled, folded, and plaited. Between the two was a layer of connective tissue so loose in its arrangement as to resemble a mass of fluffy cotton. One purpose of these structures is evidently to allow for the expansion necessitated by the growth of the embryos. The eggs lay separate from each other in spindle-shaped depressions or compartments. Each egg was encased in a shell of very thin but tough and elastic material, highly iridescent in appearance and curiously wrinkled and plaited at the ends.

Unfortunately, no note was made as to whether the heads pointed forward or backward. The heads must grow proportionately to the growth of the body.

But because of their bluntly rounded shape, head parturition must be at least fairly easy. It is unfortunate that no camera was at hand and no photographs made.

III.—From Beaufort—1912–13–14. Chronologically the next reporter is Radcliffe (1916, pp. 265–6, pl. 44, fig. 1), long-time director of the U.S. Fisheries Laboratory at Beaufort. He found the shovelnosed shark not uncommon in the waters of Beaufort and Morehead and lists 16 taken in 1912, 13 and 14.

In a gravid female 58 in. long, taken at Beaufort, he found 6 foetuses in the left uterus and 3 in the right. These ranged from about 4 to 4.5 in. long. From another pregnant female about 50 in. long, Radcliffe got 4 foetuses from each oviduct. These ran from about 7 to 7.5 in. long. Their rounded heads pointed forward. Each was attached by a yolk-stalk placenta to the uterine wall. Radcliffe's figure of a foetus is reproduced herein as Fig. 3. It is the only figure of the young of this fish made from a photograph, so far as this search shows.

Finally, Bigelow and Schroeder (1948, p. 424) say that the Museum of Comparative Zoology, in Cambridge, Massachusetts, U. S. A., has a female *Sphyrna tiburo* 43 in. long that contained 8 young, each about 12 in. long, "nearly ready for birth." This, then, seems to set an approximate size for parturition. So far as can be found, parturition has never been witnessed, but it seems fair to say that it will be by "head presentation," since in that way there is least resistance. Furthermore, it is probably fairly easy, as may be seen in Radcliffe's photograph (Fig. 3). But what about the wide hammerhead?

Zygaena laticeps (*Sphyrna blochii*)—a Normal Hammerhead

The shark designated by Cantor as *Zygaena laticeps*, is synonymized as *Z. blochii* by Day, and as *Sphyrna blochii* by Setna & Sarangdhar. All these names apply to one and the same species. The last designation is presumably the correct one.

T. E. Cantor, as early as 1845 (pp. 272–4), had already cleared up in a general way the matter of parturition in this shark with the wide hammer. There had come to him a gravid female taken off the coast of Malaya. This fish, which Cantor called *Z. laticeps* (wide head), was 4 ft., 4 in. long over all and the head was 2 ft., 1½ in. in breadth along the anterior edge of the hammer. From her were taken 18 young each measuring 1 ft., 1½ in. in length and with the head 6 in. wide over the hammer. This statement of 18 young in the two oviducts must be erroneous if each was 13½ in. long and had a 6-in. wide hammer. One cannot understand how these 18 could have been contained in the abdomen of a female only 4 ft., 4 in. long over all. However, as regards the heads, Cantor says:

> The lateral processes of the head were in the foetus considerably bent in a backward and inward direction, imparting to the head the shape of a broad arrow, whereas the processes after birth form with the body a right angle, which makes [the head of] the fish resemble a [blacksmith's] double [headed] hammer or the [capital] letter T. In the foetus the lateral processes are entirely membranous, except a narrow cartilaginous cylinder which encases the optic nerves,

and which is so pliable that it does not prevent each lateral process from lying in close contact with the sides of the body, and thus they take up but comparatively small room and offer no obstacles under parturition. . . . The smallest specimen which I observed of *Z. laticeps* in its perfect [free-swimming?] state, I mean in which the lateral processes of the head form a right angle with the body, measured 1½ ft. in total length. From this I infer that the lateral processes

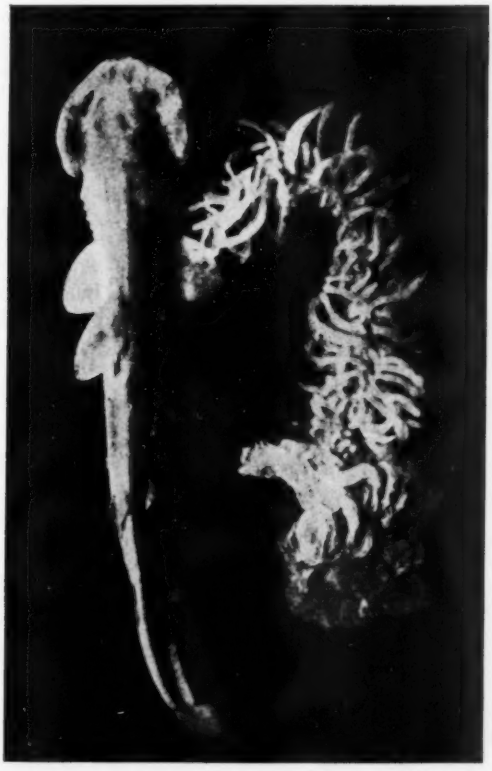

FIG. 3. Intra-uterine foetus of *Sphyrna tiburo* seen from above. Note the much rounded and evidently soft hammer-lobes, which would offer little resistance in parturition. Note also the yolk cord loaded with appendicula, and at the outer end the enlargement of the cord—a functional placenta. (After Radcliffe, 1916.)

of the head, which in the foetus branch off from the head at a sharp angle, pointing backwards, change their position and acquire firmness at no remote period after birth.

The observation of the flexible hammer is of the highest importance and clears up what has long been a mystery. First of all it makes possible the packing of the embryos in the uteri of these small sharks. However, one must query the possibility of 18 foetuses in the abdomen of a 4-ft., 4-in. mother. But Cantor certainly

does explain how parturition is possible. And this can only be by "head presentation." Unfortunately he gives no figure of a foetus with the laid-back optic lobes—the heads of the hammer.

Day (1888, p. 179) reduces *Z. laticeps* to synonymy as *Z. blochii*. He figures a young (free-swimming ?) specimen 17.5 in. long (1888, pl. CLXXXIX, fig. 4) in lateral view, but although he must have read Cantor's paper (which he lists in his references) he does not refer to the flexible lobes of the hammer in the foetus. If only his figure had been in ventral view, it would possibly have shown the yolk stalk (umbilical) scar, and have given evidence of the size attained at parturition.

Two Indian zoologists, Drs. S. B. Setna and P. N. Sarangdhar of Bombay, have prepared an extensive paper—"Breeding Biology of Bombay Elasmobranchs." This is in press in "Records of the Indian Museum," but publication has been long delayed. In this condition of affairs, Dr. Sarangdhar (with his editor, Dr. S. L. Hora, in full accord) has kindly sent me a copy of the text with permission to use any of the data therein contained. Later Dr. Sarangdhar sent me photographs of a gravid female of *Sphyrna blochii* (which name they prefer) and of the embryos with laid-back hammers. It is difficult to make sufficiently strong acknowledgment of these data, which are invaluable for the present article.

In Fig. 4 is portrayed a gravid female *Sphyrna blochii* from Bombay waters. She was 4 ft., 5 in. long "over all" and 1 ft., $11\frac{1}{4}$ in. wide over the hammer. She had 4 foetuses in each uterus, each foetus being 1 ft., $3\frac{1}{2}$ in. in total length. Note the left side of the abdomen swollen because of the four foetuses in the left uterus. On the right the shadow hides most of the enlarged abdomen. Notable are the very long and slender lobes of the hammer. Compare them with the lobes in Fig. 1.

With the kind permission of Drs. Setna and Sarangdhar, the following extensive data are taken from their MS. Their value will be apparent to the reader.

Of *Sphyrna blochii* they had 17 females, ranging from very early pregnancy to a "post pregnant condition." These varied in length from 3 ft., 5 in. to 4 ft., 8 in., the average being about 4 ft. These sizes of adults confirm Cantor. The number of foetuses in the two uteri ranged from 3 + 3 (6) to 5 + 4 (9), there being ten having 3 + 3 (6). These counts in 16 pregnant females absolutely negative Cantor's count of 18 young in his 4-ft., 4-in. female. His 18 must be a typographical error for 8. The Bombay foetuses, according to the stage of pregnancy ranged from 43 mm. (1.7 in.) to 450 mm. (18 in.) in length, the majority being in the 300 + mm. (ca. 12 in.) class.

Since our Indian authors treat the breeding biology of 12 other sharks and 7 rays in the same thorough way that they do *Sphyrna blochii*, it is readily seen of what great value their thorough-going studies of all these fishes will be when they are published and widely distributed.

Of particular interest to this paper on parturition are their three statements concerning the development of the hammer lobes in the young of *S. blochii*. In

a 70 mm. (2.8 in.) embryo—"Each oculo-narial expansion [was] shorter than its width." In a 287 mm. (11.4 in.)—"Oculo-narial expansion curved backward to give a horseshoe shape to the head region." In a 362 mm. (14.5 in.)—"Oculo-narial expansions still directed posteriorly." For this marvelous preparation for parturition, see Fig. 5.

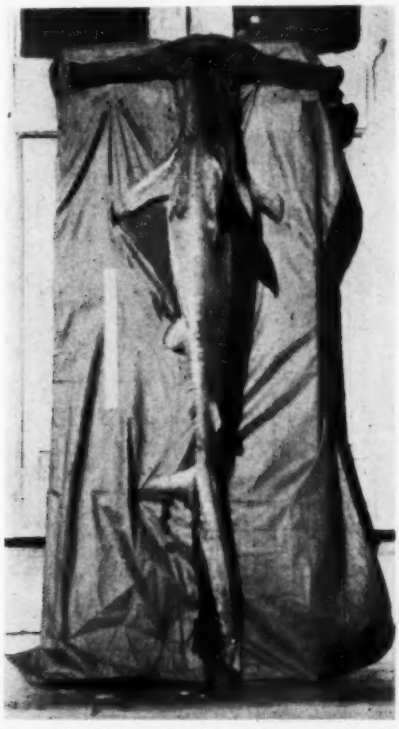

FIG. 4. A gravid *Sphyrna blochii* from Bombay. She was 4 ft., 5 in. long over all and the slender hammer measured 1 ft., 11¼ in. in width. Note the enlarged left side of the abdomen —swollen with four foetuses (each 1 ft., 3½ in. long) in the left uterus. (Photograph from Dr. Sarangdhar)

Setna and Sarangdhar had a number of free-swimming young 450 mm. (18 in.) to 525 mm. (21 in.) long "with fresh umbilical scars." From this it is clear that Cantor's 13½-in. foetuses in the maternal uteri were far from parturition. The great value of these data concerning viviparity and parturition in the hammerhead shark, *Sphyrna blochii*, is apparent on the face of it. Here are exact measurements of gravid mothers and of their foetuses, and of two just-born and free-swimming young. The photographs of these structures (Figs. 4 & 5), the first ever published, afford the ocular proof lacking in Cantor's account.

PARTURITION IN THE SPINY DOGFISHES

The spiny dogfishes have the first and second dorsal fins each provided with a frontal backward-pointing spine—the posterior one being the longer and better

FIG. 5. Two foetal *Sphyrna blochii*, each 1 ft., 3¾ in. total length and with 6-in. hammers laid back along side of body to facilitate parturition. The right specimen is in dorsal, the left in ventral aspect. Note remains of yolk stalks and 1 placenta. These are the only known figures showing the bent back hammer lobes. (Photograph from Dr. Sarangdhar.)

developed. Such is the armament of the European and the American fish. Although these spines point backward, it is easily understood that, since the fish is viviparous, they would lacerate the uterus in which the rather active young are held, and probably during parturition would do the same thing to the external opening of the generative organ as they pass out. Thus a protective device is needed and such has been referred to by several authors.

Squalus acanthias (*Acanthias vulgaris*)

The spiny dogfish of the Eastern-and-Western-North Atlantic is one and the same fish, and what follows here in the matter of protection of the mother at parturition holds, of course, for fish from the West as well as from the East.

As early as 1847, Robert Ball described at the meeting of the Royal Irish Academy (but unfortunately did not figure) the protective device on the spines of the foetus. Here follows the secretary's account of Ball's oral description of this device (1847, p. 230):

> Mr. Ball brought under notice of the Academy as an observed fact, a beautiful provision in the foetus of the spined dog-fish (*Acanthias vulgaris*), by which the mother is protected from being lacerated by the spines of the young before and at birth. He exhibited two perfectly developed young which he had taken from the mother on the 30th of November last. In these the spines were each covered at the point with a small knob of cartilage, fastened by straps of the same material, passing down, one on each of the three [free] sides [front and two sides] of each spine, in such a manner as evidently to become easily detached at birth, thus allowing the little animal to commence life effectively armed.

Spinax niger (*Etmopterus spinax*)

This deep-water shark with dorsal spines is found in the Atlantic west of Europe. In 1901 Koppen published a paper based on a histological study of the development of the fin spines. His specimens of the baby fishes were *ca.* 3.5 cm. (1.4 in.), 5.5 cm. (2.2 in.), 7.5 cm. (3 in.), and 11.5 cm. (4.6 in.) in length. At the apex of the spine in each stage was a pinhead structure, a small knob or protective cap. During the developmental stages of the fin spines, the protective covering is always better developed on the longer posterior spine than on the smaller anterior one. When the foetus is extruded into the water, the cap softens up and drops off. This cap is shown in the Koppen's text-figure on his page 508, which is reproduced herein as Fig. 6. The figure and the caption make the matter clear without further words.

There are various forms of spiny sharks found in the Western North Atlantic, but for none of these have any accounts been found of devices protective of the mother in parturition. For the other account of such we must go to the far-off waters of New South Wales, Australia.

Flakeus megalops, the Australian Dogfish

Of this Australian piked dogfish, whose spines likewise point backward, Whitley (1940, p. 138, fig. 138) in the caption to his figure says, "The dorsal spines of the embryo [piked] dogfish are padded with knobs to protect the mother." And in his description of the fish he writes: "Ovoviviparous: The dorsal spines of the embryo are tipped with pad-like knobs so as not to lacerate the mother". But unfortunately his figure of the embryo is too small to show the knobs. If only he had had a spine and its knob figured in proper enlargement it would have added much to his book and to this article.

There seems to be no direct observation of parturition in any other spiny

dogfishes, but the backward-pointing spines plainly indicate birth by "head presentation," and it is a fair presumption that knobbed spines will be found on the foetuses of all forms of spike-finned sharks.

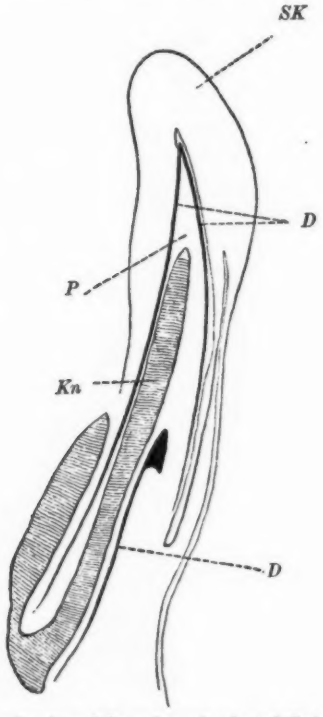

FIG. 6. Vertical section of a dorsal fin spine of a foetal *Spinax niger:* SK, cartilaginous cap over the top of spine; D, dentine (bone) of the spine; D, (below) dentine of a replacing spine?; P, pulp cavity; Kn, cartilaginous fundament of spine. (After Koppen, 1901.)

PARTURITION IN FORMS INTERMEDIATE BETWEEN THE SHARKS AND THE RAYS

We are now come in our parturition studies to two "sharks" which are aberrant forms transitional between the normal sharks and the normal rays. We will first study the more shark-like—*i.e.* the lesser ray-like form.

Pristiophorus cirratus, the Saw Shark

This is a transitional shark, one with the slender shark body but with the head and snout produced into a long blade-like implement beset with teeth implanted in sockets in the right and left edges. It has two long feelers (lacking in the sawfish) inserted below about midway of the blade (Fig. 7 herein). Hence the specific name *cirratus*. Most sharks have the mouth more or less ventral—placed under

the snout. But in the saw shark, the snout is produced into a long saw that gives the mouth a decidedly ventral—under the head—position. The spiracles are lateral and the gill slits sub-lateral (Fig. 7). These organs are in transition.

The intra-uterine young of the viviparous saw shark also has a saw and the reader is already saying that it must, in its intra-uterine activities, fearfully lacerate the mother, to say nothing of what it will do to the cloaca on its outward passage. But, as will now be seen, Nature has provided for this contingency.

In 1851, Bicheno (pp. 223–5) described a female saw shark taken in the Derwent River, Tasmania. She was only 5 ft. long over all, but in her uterus were found two young, one 6 and the other 5 in. in length over all. Unfortunately they were not figured. They were judged to be about ready for parturition. How this was provided for is thus stated by the author: "The Spines on the snouts of these young ones, although standing at right angles with the edges, are easily laid back close along the sides of the weapon, so as to offer no resistance to its passage through any channel where the head would pass."

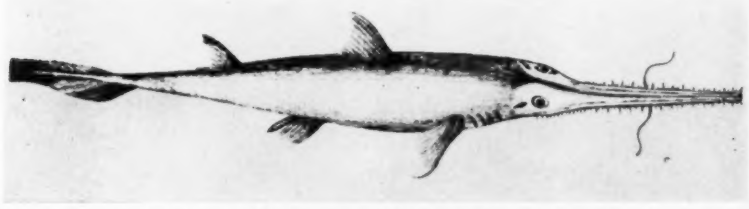

FIG. 7. The Saw-Shark—*Pristiophorus cuspidatus*. Note beak with spines long and short, and the two cirri. The eyes and spiracles are lateral, the gill slits sublateral. This fish is a transitional form between sharks and rays. (From Whitley after Latham, 1794.)

This recalls the manner of folding back the "hammer" of the baby *Sphyrna blochii* to facilitate parturition.

Fifty-five years later, D. G. Stead (1906.1, p. 425) exhibited a mature intra-uterine foetus of the little saw shark, *Pristiophorus cirratus* (Latham) and pointed out that an examination of the rostral lamina or "saw" in the mature foetus of *Pristiophorus cirratus* revealed the highly interesting fact that it was armed on each side, at regular intervals, with long "spines" only; there being none of the small intermediate spines which are characteristic of the saw in the adult (Fig. 7) or in the half-grown specimen exhibited. It was interesting to note also that these teeth were not fixed rigidly at right angles to the "saw" as in the adult, but were soft, movable, directed backward and lay pressed against each side, thus protecting both foetus and mother during parturition. Unfortunately a figure is lacking.

This report by Stead was reproduced verbatim later in the year in another journal (Stead, D. G., 1906.2, p. 822).

Whitley (1940, pp. 154–5) has done more to clear up the means of parturition in the saw shark than anyone else. He has figured the shark and its rostral

apparatus very carefully. His figures and explanations now follow. Fig. 7 herein is a photograph of Whitley's reproduction (his p. 154) of Latham's original drawing of an adult published in 1794. Note the teeth of two kinds set in the edges of the jaw and the two cirri set at about the middle of the under side of the saw.

The statement about the two kinds of teeth in the saw of the adult *Pristio-*

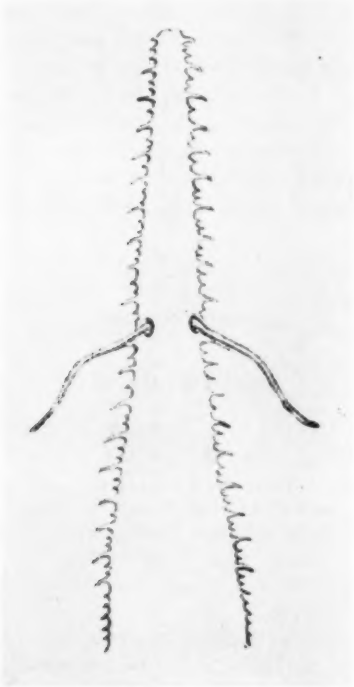

FIG. 8. Under side of saw of an adult *Pristiophorus cirratus*, showing cirri and backwardly pointing teeth of two kinds implanted in the edges of the saw. (From Whitley, 1940).

phorus is made clear by inspection of Whitley's figure of the saw of an adult seen from below (his figure 6, p. 155). Its reproduction as Fig. 8 herein shows the large teeth bent slightly backward. Between these are the small teeth, which plainly would cause little if any trouble in parturition.

This is made clear by Whitley's drawing (1940, fig. 1, p. 155). Fig. 9 herein shows a 12.5-inch foetus from the dorsal side with the rostral teeth folded back along the edge of the saw and out of sight. From the dorsal view it is clear that these lateral saw teeth cause no trouble in parturition. Fig. 10 (a reproduction of Whitley's figure 2, p. 155) further emphasizes the fact that these rostral teeth of the foetal Pristiophorus, being bent downward and backward (a most re-

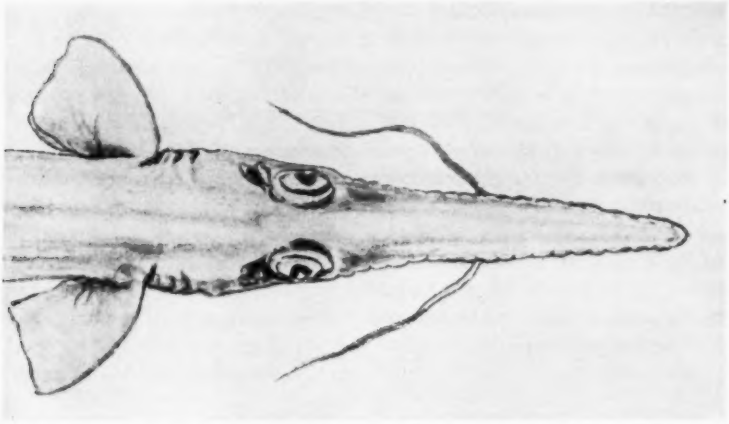

FIG. 9. A 12.5-in. foetal *Pristiophorus cirratus*, showing teeth laid back and out of sight to facilitate "head presentation" parturition. (After Whitley, 1940.)

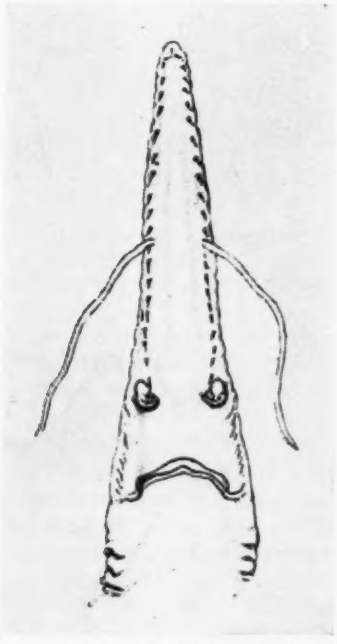

FIG. 10. Underside of saw of a 12.5 in. foetal Saw Shark showing the teeth bent backward to facilitate parturition. (After Whitley, 1940.)

markable adaptation) give no trouble whatever in parturition. This in the shark can only be by head presentation.

The next elasmobranch whose parturition will be studied is in structure less a shark than the saw shark is. It is plainly a shark further on the way to becoming a ray.

Pristis pectinatus, the Sawfish

The sawfish, as Fig. 11 shows, is another intermediate form. Posteriorly it has the general shark form of *Pristiophorus*, or any rather long-bodied shark. But anteriorly the head and body show ray-like characters in that they are somewhat flattened dorso-ventrally and somewhat expanded laterally; the expanded pectoral fins, while free from the head, are rather short and have broad bases measuring about two-thirds of the length of the fin. Other ray-like characters are the dorsal eyes and spiracles and the ventral mouth and gill openings. Notable

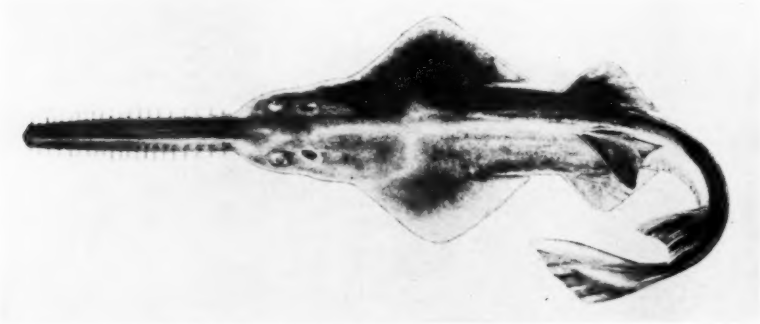

FIG. 11. An adult Sawfish, *Pristis pectinatus*. Note the flattened head and anterior body, and the wide pectoral fins; also the dorsal eyes and spiracles and ventral mouth of this ray-shark. The saw is beset with bony teeth solidly implanted in the sides of a bony snout. (After Garman, 1913.)

is the snout, which is produced into the long saw beset on the edges with the strong teeth solidly set in the grooves on the edges of the rostrum. This saw sometimes attains a length of 5 or 6 feet.

When one learns that the viviparous sawfish grows to 15 or 18 feet—in comparison with the 5 or 6-foot *Pristiophorus*—one expects larger and more active embryos with better developed saws in *Pristis*. And so one finds. Then one wonders how a foetal sawfish with strong teeth set solidly in the saw, can be carried in the maternal uterus and be brought to parturition without fatal lacerations to the mother. But again Nature has made a provision and indeed a remarkable one, as we shall see.

The earliest reference to and figure of a foetal sawfish seems to be by Marcus Elieser Bloch (1786, p. 56, pl. 120). His colored figure (Fig. 12 herein) is a lateral view of a foetus with a large non-absorbed yolk-sac. Bloch does not record the source of his specimen. Although this figure shows little other than the form of the foetus, it emphasizes the flexibility of the saw in this stage. Note that the

forward end (about one-third of the total length) is bent upward and backward. This shows the extreme flexibility of the saw at this stage. The original figure is five and five-eighths of an inch long from tip of the tail to the bend of the saw. The teeth are visible to the naked eye only in the upturned section, and even these are not covered by any visible "skin." It does not seem worth-while to devote more attention to this figure and account since better ones are at hand. This figure (enlarged and) identical with that noted is found in Bloch's folio "Ichthyologie", Berlin (1787, p. 38, pl. 120). The plates in these volumes are identical.

The first investigator, so far as this search has revealed, to dissect a pregnant female sawfish, was Thomas Southwell. His account (1910, pp. 137–9) is quite brief but most valuable. His specimen was taken in four fathoms in Portugal Bay, Ceylon Pearl Banks, on Dec. 15, 1909. This great fish was $15\frac{1}{2}$ ft. long and $5\frac{1}{4}$ ft. wide over the pectorals, and her weight was "approximately 170 lbs."—which is surely a notable underestimate.

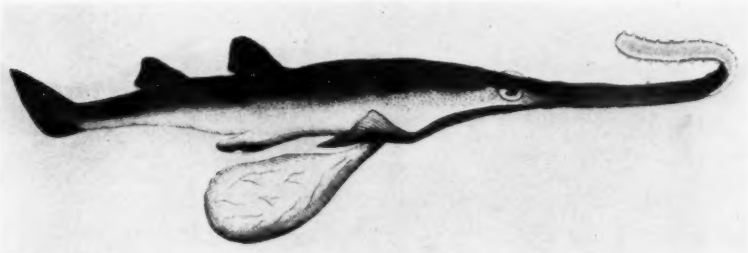

FIG. 12. The first published figure of a foetal sawfish. The bent tip of the saw, the barely visible teeth in this tip, and the still large yolk sac—all testify to the extreme youth of this foetus. There is no trace of a sheath over the saw. (After Bloch, 1786.)

In this pregnant female, the much enlarged oviducts (uteri) were 17 in. long by $15\frac{1}{2}$ in. in circumference. The right uterus contained 11 foetuses, the left 12. "These oviducts were united at the cloaca to form a wide infundibular opening." Southwell further states that:

> The embryos themselves measured in extreme length 14 in.; breadth across the pectoral fins 5 in.; length of rostrum 5 in.; length of placental stalk 5 in.; diameter of yolk sac $2\frac{3}{4}$ in. With reference to the rostrum, the dentition, while apparent, was obscured by being entirely covered by a transparent cartilaginous tissue, which of necessity must disappear later.

For all these things, see Fig. 13 herein. This is a photographic copy of Southwell's poor figure made from a poor photograph. But it is the earliest representation of the foetal sawfish with its shielded saw and teeth found in this search. When and how the sheath is formed are problems still awaiting solution.

With 11 foetuses packed in one uterus and 12 in the other, these oviducts and the abdomen must have been enormously distended. Southwell notes that: "The embryos all lay horizontally, i.e., parallel to the axis of the parent. . . . Some em-

bryos lay with the rostrum close to the cloacal opening, whilst others were exactly opposite." Elsewhere (his p. 138, bottom) Southwell makes this (to us) interesting notation: "Immediately on capture [of the pregnant female] a discharge occurred from the cloaca, consisting of a serous fluid tinged with blood, and two embryos were noted protruding, rostrum presenting." These, however, being encumbered with large yolk sacs were plainly not near parturition. This slight protrusion of the saws of the immature foetuses, in my judgment, resulted

FIG. 13. A more advanced stage of a foetal sawfish (from Ceylon) seen from below. This is the earliest figure of a young *Pristis pectinatus* showing the sheath over the saw. Note the dependent yolk sac. (After Southwell, 1910.)

from a reflex action on the uterine muscles of the pain felt by the mother in the capture or dissection. Further data on such reflexes will be presented later in the section on Rays.

This setting forth of Southwell's badly scrambled account shows that while he is not the discoverer of viviparity in the sawfish, he is the first to see and figure the sheath over the rostrum. But since he does not emphasize the function of the rostral sheath, it is clear that he did not realize the interest and value of his discovery in this fish. Yet this sheath-covered saw in the sawfish is the most remarkable adaptation for parturition found in any shark studied.

Southwell sent three of his sawfish embryos to the American Museum. Hussakof, at that time Associate Curator of the Museum's Department of Fishes, had

two figures drawn of one of these foetuses (355 mm.—14 in. total length). One figure is a lateral view and the other of the little fish in ventral aspect. The latter is reproduced herein as Fig. 14. Note the excellent representation of the saw of the little fish. Hussakof states that it was 117 mm. (4.7 in.) long and "still flexible" despite its long sojourn in alcohol. He also notes that "The teeth are completely enveloped in membrane, their points forming a series of slight crenulations at the margins of the rostrum. On holding the saw to the light, they can be clearly seen; they are yellowish, sharply pointed and average 6 mm. in length. . . . The toothless basal portion of the saw measures 22 mm. or about one-sixth of the entire saw." Hussakof concludes with a complete table of measurements of the embryo. He, like Southwell, failed to perceive the special function in parturition of the sheath on the saw.

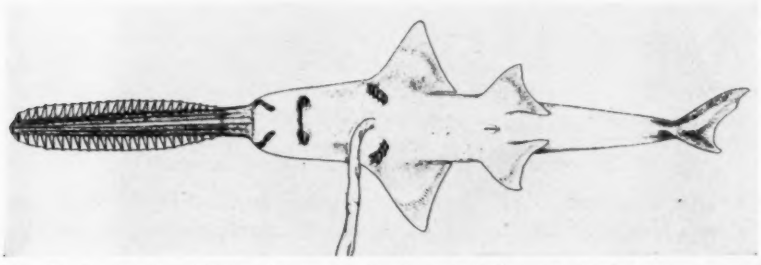

FIG. 14. A second and better figure of a foetal sawfish (from Ceylon) in the same stage as the preceding. Note the saw and teeth covered by the transparent sheath. The outer end of the yolk stalk and the large yolk sac, well shown in Fig. 13, have been cut off the figure. (After Hussakof, 1912.)

Two other writers may be briefly quoted. H. M. Smith (1907, p. 39) notes that "The young are able to swim at birth, although the saw and the teeth are at first soft like leather." And Whitley (1940, p. 178) remarks of *Pristis zijsron* Bleeker that it is " . . . doubtless viviparous, since its allies in other countries have young with the rostral teeth sheathed before birth."

Last of all, Setna and Sarangdhar in their MS. previously referred to, say of an 8-ft., 11-in. gravid sawfish with three uterine embryos, 537 mm. (*ca.* 21 in.) long, "Parturition stage; teeth on saw not yet through the membrane enveloping them. The lateral teeth of the 'saw' are cut only after the assumption of free life." This indicates fairly clearly the apparent size of the young at birth.

A few last words must be added. Southwell's finding in the cloacal region of his dead pregnant *Pristis* two young with large yolk sacs does not indicate parturition. In all known cases of actual parturition in elasmobranchs, this does not come until all yolk is absorbed and the young are ready to fend for themselves. However, parturition in the sawfish would seem facilitated best by head presentation.

The saw shark and the sawfish are the transitional forms between sharks and

rays which are known to me to be viviparous, so far as this study has found. From *Pristis pectinatus* onward, the intermediate forms become steadily wider forward by an expansion of the head and particularly of the pectorals and their bases—and in some forms of the pelvis also. Since the true rays, into which these forms grade, are all viviparous, so far as known, one must think that at least some of the transitionals are also viviparous. Furthermore, since the young will evidently be broad in the head and pectoral region, one may expect at least difficult parturition. Possibly the facilitation of birth in these fishes is something like what will presently be found in the normal rays. However, it is clear that here is an unexplored field which will yield interesting returns to the investigator.

From the transitional forms just considered, we now go forward to the Sub-Order Batoidei—Rays. This group seems at the present to be somewhat of an "omnium gatherum" for the systematists, since in it are included the intermediate forms considered above and others which in general appearance and make-up would surely seem to be "intermediates" between the sharks and the more specialized Batoidei—the Rays proper. Herein we shall study parturition in rays in the narrower sense—in normal rays.

PARTURITION IN THE TRUE RAYS—BATOIDEI

The true Rays, speaking generally, may be described as Elasmobranchs which have been shortened longitudinally, flattened dorso-ventrally (vertically) and expanded laterally. Thus they are fashioned about equally on two axes—fore and aft and right-left. They may be roughly divided into Skates, which are bottom dwellers and are oviparous, and Rays proper which are free-swimmers and are viviparous. Our interests are in the latter.

In the Rays (in the restricted sense) the body is composed of the flattened head and trunk, and the sides of the greatly expanded pectorals, which are confluent with the head and body. Thus the body is disc-shaped or rhomboidal. In the whiptailed and eagle rays, the pectorals are pointed and wing-like and in these forms the transverse diameter of the body is greater. In them the slender rat-like tail is long (often longer than the body)—whiplash-like. In all the rays, the spiracles are dorsal and the mouth and gill openings are ventral. A typical whip-tailed ray, *Rhinoptera bonasus*, is portrayed in Fig. 15 herein.

When a student sees a ray and grasps the import of the transformation, he must realize that, in comparison with the shark's abdominal cavity (greatly restricted when compared with that of the higher viviparous animals), the abdomen of the ray is reduced to the limit. This is confirmed by dissection. And then the student asks how can viviparity (pregnancy and parturition) be effected unless the young at birth are of insignificant size and are comparatively helpless.

Young rays, like all young viviparous animals, are born of some size, and are complete enough in their organs to be able at birth to fend for themselves. In fact, the size of these young, flat, wide-pectoral raylets, when ready for birth, is the thing that makes their parturition a matter of seeming impossibility. Some comparative measurements of mothers and foetuses are very pertinent just here.

COMPARATIVE SIZES ATTAINED BY UNBORN RAY EMBRYOS

This matter of sizes of ray foetuses is fundamental in viviparous rays and deserves careful consideration. So far as this search reveals, the first investigators to record the sizes of the unborn baby rays, in comparison with the sizes of the mothers, were Wood-Mason and Alcock in India. These records will be studied first and by genera and species.

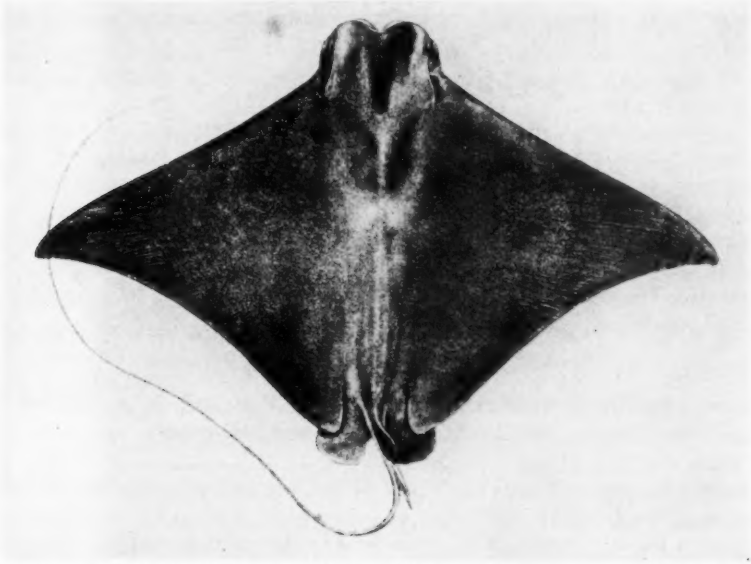

FIG. 15. An adult female Cow-nosed Ray—*Rhinoptera bonasus*—frequently referred to herein for various activities. Note the bilobed blunt nose, the pointed pectorals, and the long whiplash tail with spine at its base. (From Garman, 1913.)

In India

Trygon bleekeri.—First of all Alcock early (1891, p. 53) dissected "a female with a disk of very large dimensions" without giving measurements. In her he found that "the right oviduct was enormously dilated and contained . . . a male foetus with a disk 11.75 in. long and 10.75 in. broad."

Trygon walga.—Of two parturient females of this ray, taken on April 8, 1891, Wood-Mason and Alcock write thus (1892, p. 205):

> The abdomen in one of them was so much distended that the normal flat shape of the fish was obscured. On transfer of this specimen to a bucket [tub?] of sea water, two young ones were seen to be suddenly extruded from the cloacal orifice—one a few minutes after the other. The young ones swam about vigorously in the bucket. Relative to the size of the abdominal cavity of the mother, which in this species is much contracted, the young ones are enormous, and, seeing the two of them beside the opened maternal abdomen, it appeared almost incredible that they could ever have been compressed into such a confined space.

Then they continue with these specific data. "The mother was 205 mm. long to end of ventrals, the embryos were each 67 mm. long. The breadth of the mother was 185 mm.; of embryo No. 1, 70 mm.; of No. 2, 72 mm." Thus the foetuses were about one-third the size (area) of the mother. Relatively they were "enormous."

In this matter of the relative size (area) of the foetal ray and the mother, some observations made by the writer at the Beaufort, North Carolina, Laboratory of the U. S. Bureau of Fisheries in the early 1900's are pertinent and will be quoted.

At Beaufort, North Carolina, U. S. A.

Rhinoptera bonasus.—On May 31, 1909, a cow-nosed ray (Fig. 15) 24 in. wide, on being clubbed on the head in the small boat to keep her quiet, gave birth to two young, each 8.5 in. long (tip of nose to end of ventral fin), and 13.5 in. wide (Gudger, 1910, p. 397).

Dasyatis say.—On June 17, 1910, a female common sting ray was taken. She was 36 in. wide by 35 in. long. From her were obtained two young of about equal size. The one measured was 14.75 in. wide and 5.75 in. long. In addition the tail was 9.5 in. long (Gudger, 1912, p. 145).

In Various Other Regions

Several rays reported from far-scattered localities attain large size and bear large, wide embryos. Definite data for certain ones are pertinent and will now be set forth.

Trygon thalassia.—This is the largest of the New Zealand sting rays. Seymour George writes (1881, p. 426) that he captured at Kawau an unusually large female—4 ft., 7 in. broad, 3 ft., 11 in. long to root of tail; over-all, 9 ft., 11 in. Weight estimated at 200 lbs. Dragged on shore she gave birth to a foetus 1 ft., 4 in. wide, 1 ft., 1 in. long to base of tail; over-all length 3 ft., 3 in. Its spine was 2 in. long. This trygon was a large sting ray, but in the waters of Australia is found a related form, *Bathytoshia brevicaudata*, that is probably the largest stingaree in the sea. I have a photograph of a female estimated at 6 or 7 ft. wide and about 14 ft. long including the vastly long tail, the base of which is about as large as a man's leg. It is literally a colossus among stingarees, but it had no embryo and one can only conjecture how enormous such would have been.

Before leaving the subject of relative sizes of embryos and mothers in the Batoidei, it may be of interest briefly to call attention to the giants of all the rays, the widely distributed non-spine-bearing Mantidae. The best known member of this group attains an ultimate size of 20–25 ft. over its pointed "wings." It is viviparous, but until recently there has been little knowledge of the literally enormous size of its embryos.

Manta hamiltoni.—This form is one of the giants of the Mantidae. Here are the very exact data that Beebe and Tee-Van (1941, p. 275) give of a pregnant adult taken at the Galapagos Islands in June, 1925.—Width, 18 ft.; weight (cut

up and the parts weighed separately), 2310 lbs. (probably 100 to 200 lbs. of blood and other body liquids were lost in the process). They took an immature embryo from the body of the mother. It weighed 28 lbs. and measured 45 in. wide. It will be described later.

After reading this presentation of amazing facts, the reader is asking "How can a young ray—such as Wood-Mason and Alcock (1892, p. 204) call 'enormous' (in proportion to the size of the mother) and to say nothing of the 45-inch wide and 28-pound heavy (literally enormous) foetus of the *Manta* just noted—be passed out through the distensible but still restricted genital and cloacal orifices of the mother?" The question is one of surface area of the foetus, due to great spread of the pectoral fins.

To help understand this extraordinary phenomenon, let us suppose two men are in adjoining rooms separated by a wooden partition of stout boards intact save for a knothole in one of the boards. A has an important document on heavy 8 by 10 in. paper which he wishes to pass to B "as is" (without tearing or folding). He rolls it up tightly (one edge in and one out) and passes it through the knothole.

And now we shall see that Nature does just the same thing with these relatively large and wide-winged baby rays in the matter of viviparity.

FOR PARTURITION EACH RAY FOETUS IS ROLLED UP ON ITS LONG AXIS

And so, difficult as it may seem, Nature makes a provision in the matter of parturition of the wide ray embryo. She teaches the baby ray to roll itself up tightly on its long axis, thus reducing its gross surface. Then the flexible cigar-shaped body, well lubricated by the milky uterine fluid in which it is immersed, slips out, small end foremost (?), through the distended orifice into the sea, unfolds its wings and swims about, as Wood-Mason and Alcock noted (1892, p. 204).

Single Reports of Rolled Up Foetuses from Various Regions

French Guiana.—The earliest record that has come to light of this method of facilitating parturiton in rays is from the pen of Galard de Terraube (1799, pp. 101–2). He says that on the coast of Cayenne a pregnant female ray was caught and suspended by a hook. This unusual position and the pain therefrom hastened the expulsion of four embryos, one after the other. Each was rolled like a "gauffre" (a French pancake rolled with one edge in and one out, like a sheet of paper). When the young were dropped into the water, each opened its wings and swam away.

Jamaica, British West Indies.—*Mobula olfersi*, the devilfish of Jamaica, is the next ray recorded with a rolled up or folded embryo in the mother's uterus. The foetus was taken from a pregnant female at Jamaica and figured and described by Hill (1863, pp. 175–6). Hill confused his small Mantid ray with the gigantic *Cephaloptera massenoides* (*Manta birostris* ?) of these same waters, as two of his

figures indicate. However, his ray was pretty surely the smaller *Mobula olfersi*. Hill speaks of its having pointed horns on its head made by the anterior flaps of the pectorals (the cephalic fins) which are rolled up and "twisted like a coiled leaf with a twirl that fashions into a groove." This gives the ray the appearance of having pointed twisted horns (as I have seen them) and identifies it as *Mobula*

FIG. 16. The uterine foetus of the small Devilfish of Jamaica (*Mobula olfersi* ?). Note the rolled up cephalic fins and also the wide pectorals folded over the back—the earliest representation of such. (From Hill, 1863.)

olfersi. This mantid ray was small: 4 ft. wide and 2 ft., 1½ in. long to the dorsal fin at root of tail. This, too, indicates *Mobula*, not *Manta*.

This specimen was "a gravid female having within it a foetus just mature for extrusion, 16 in. broad." Hill (1863, p. 176) figures the foetus—the first rolled or folded embryo ray ever immortalized in a drawing. "The flukes of the [pectoral] fins were folded back over the back, lapping one another thus:—[Fig. 16 herein] The fins when extended were 16 inches across, and the length of the body from the head to the tip of the tail [was] 24 inches." Note the size of the body of this embryo. It is in keeping with the size and solidity of the body of the adult mother.

Galapagos Is.—Manta hamiltoni.—This ray, a giant of its tribe, was captured

by Beebe and Tee-Van in 1925 off Narborough I. It was an 18-ft. pregnant female. They reported (1941, p. 275) that she had in her a huge male embryo 45 in. wide and 28 lbs. in weight. They say further of this specimen: "On the date of capture (June 11, 1925) the embryo had its tail protruding from [the cloaca of] the mother [a very significant statement] and the yolk was almost completely absorbed; hence it would probably have been born in a day or two. The yolk sac remained as a knotted string six inches long."

This huge foetus must have been practically "ripe" for parturition, and it is really extraordinary (in the light of certain facts to be presented later) that it was not lost in the fight of the capture. Its preservation was most fortunate, also, as will now be seen, for a photograph of it was made.

Beebe is at this writing in Trinidad, but Tee-Van (at the N. Y. Zoological Park) was asked about how the pectoral fins were folded. He answered "over the back," and said that a photograph of the embryo was made. Later he had a print made from the negative. This photograph shows not only the large "wings" laid back over the dorsum of the baby ray and presents the white under surface, but above the wings the cephalic fins or "horns" are also so folded as to show their white ventral surfaces. This portrayal (Fig. 17 herein) is the first and only photograph of a ray embryo brought to light in this search. Note that the foetus is wide forward and narrow behind—facilitating birth by "tail presentation."

Mobula diabolus is found in Indian waters. Setna and Sarangdhar, in their MS. sent to me, list three specimens in parturition stages with the uterine mucosa produced into villi or trophonemata, and with the yolk sac and stalk of each foetus completely absorbed. On the matters of particular interest here, they say:—"Only the left oviduct and left uterus are present (no room for right one). . . . A single young one is born at a time, measuring 400 mm. [16 in.] across the disc. It lies in a supine position within the uterus with its wings folded ventrally and medially." One wishes that this were clearer. Can it be that each wing is rolled up ventrally? Such was found for another ray studied long ago at Beaufort, North Carolina, U. S. A., by the present writer, as will be noted later.

Various Indian rays have been studied for uterogestation. Those recorded as having rolled up foetuses will now be listed chronologically and the embryos will be briefly described.

Multiple Reports of Rolled Up Foetuses from India

Trygon bleekeri.—This ray is reported on by Wood-Mason and Alcock (1891, p. 363). They found a gravid female to have the right oviduct "enormously dilated" and containing a well-developed male foetus with a disk 11.75 in. long and 10.75 in. broad. "The folded foetus lay free in the uterine cavity." The word "folded" is used a second time in another place. Just what is meant is not clear, but it may be presumed that it is merely a form of rolling up like that figured for *Mobula olfersi* (Fig. 16) and for *Manta hamiltoni* (Fig. 17 herein). It is most unfortunate that no drawing was made.

Pteroplatea micrura.—The common Indian ray is the next to have its rolled up embryo described and the second to be figured. This was also done by Wood-Mason and Alcock (1891, p. 364, pl. 7, fig. 1). They say "On opening the pregnant uterus by a dorsal longitudinal incision [by which a slight cut was made in the dorsum of the foetus], the naked embryo is found lying prone, head forewards, and tightly rolled in a right-to-left spiral." This is to be seen in Fig. 18

FIG. 17. The 45-in. wide, 28-lb. heavy intra-uterine foetus of *Manta hamiltoni* has its large pectoral fins each folded back over the dorsum. Note that the cephalic fins are also folded and show the white under surfaces. This is the only known photograph of a folded foetal ray. Note also that the embryo is wide forward and narrow behind. Tail presentation in birth? (Photograph from Beebe & Tee-van.)

herein. The right pectoral is coiled in on the ventral surface of the body and the left pectoral is wrapped or "folded" to the right over the whole ventral body, and the rolled right pectoral. Thus folded, the well-lubricated foetus can pass, tail first, easily out into the sea, when it will open its fins and swim away.

In their "Further Observations" of uterogestation in Indian rays, Wood-Mason and Alcock make this remarkable statement concerning the uterine foetuses of *Pteroplatea micrura* (1892, p. 203). "We find that where, as appears to be the most common condition, two embryos occupy the [same] uterus, the one is rolled within the other—head to head and tail to tail [?]—but in such a

way as to leave the spiracles of the inner one exposed for [the villi or] the trophonemata to enter [to supply nutriment]." For this entrance of the villi into the spiracle, see Fig. 18 herein. This matter of two embryos interrolled, so to

FIG. 18. Dorsal aspect of an intra-uterine young of the Indian ray, *Pteroplatea micrura*, in the dorsally split open uterus. Note the pectorals so rolled or folded as to permit the insucking into the spiracles of the uterine villi or trophonemata. These penetrating into the stomach of the embryo nourish it with the milky fluid which they secrete. (From Wood-Mason & Alcock, 1891.)

speak, is again referred to (Wood-Mason and Alcock, 1892, p. 209). Most unfortunately, there is no figure.

This statement is as extraordinary as it is inexplicable. Nothing like it has been found in the literature nor in the personal observations of the present writer. Surely it is erroneous. The bulk of the two interrolled embryos would

offer a great obstacle to parturition—one foetus is seemingly about all that the uterine-cloacal aperture will allow to pass. If the observation is correct, it would seem that the embryos must unroll and reroll separately for passage to the external world.

This exhausts the matter of rolled up foetuses of rays, so far as this search of the older literature has gone. The writer will now give a brief résumé of his incomplete observations on rays and foetuses made at Beaufort, N. C., in the early 1900's.

Rolled Foetuses of Rays Recorded at Beaufort, North Carolina, U. S. A.

Rhinoptera bonasus.—The cownosed ray (Fig. 15) is recorded (Gudger, 1910, p. 397–398) as giving up two rolled foetuses. They are thus described: "The young are rolled each up into a tube, one pectoral fin forming the inner lining of the tube, and the other the outer—*i.e.*, each young is rolled up like a sheet of paper."

The accumulated notes of the present writer dealing with uterogestation of sharks and rays at Beaufort were published in 1912. These notes contain many details of the subject of uterogestation other than the way the ray embryos were rolled. In the absence of specific accounts, it may be taken that the rolling for all was like that of *Rhinoptera bonasus—i.e.*, "like a sheet of paper." But specific reference is made to one form and its unusual rolling up.

Dasyatis say.—The common sting ray has its embryo rolled up thus: "The young one seemingly lying on its back, turns each pectoral vertically upward and rolls it inward and downward toward the median line" (and the note adds), "like two hands with the wrists placed together, palms uppermost and fingers closed to touch palms."

My other descriptions of the rolling up are not clear enough to be quoted. Our seining crew (which the writer headed for years) caught these rays or had them given to us by our fishermen friends about five miles from the laboratory. No camera was available, the fishes had to be "worked" on the floor of our small boat and notes made amid the struggles of the rays. One ray struck at me with its tail so hard and the spine was so firmly set in a timber of the boat that the tail was cut off at the fishing grounds, and the spine left sticking in the wood until we reached the laboratory where it could be removed.*

The sharks were fairly easily handled. Struck on the nose with a hatchet, they became quiet. Furthermore, their long bodies could be stored away under the thwarts of the boat. The rays occupied more area, made a greater disturbance,

* At the time these imperfect observations were made during ten summers' work at Beaufort, the writer was collecting material, writing and securing publication for three large and important researches. Something had to stand aside, and that was the fate of the study of parturition in the rays. The writer realizes the imperfections of these observations. If some student in one of the present-day Beaufort laboratories would systematically take up the study of the parturition of rays in Beaufort and the neighboring Cape Lookout waters, a first class piece of interesting and valuable research could be done. One question that might be settled is—Do the baby rays of all kinds emerge heads or tails first?

and were more dangerous, and when knocked on the head invariably produced their young, as will be noted presently.

Before leaving these embryo rays rolled ready for parturition, two generalizations need to be stated:—I. All known true rays are viviparous. II. In all viviparous rays, parturition, so far as is known, is provided for by the rolling of the embryos into cigar-shaped bodies and their emergence is tail (the small end) first.

Now as to observations of parturition in rays, Beebe and Tee-Van record the tail of an unborn but nearly "ripe" *Manta* foetus protruding from the cloaca of the mother. And all my observations are of births tail parts first. However, the great desiderata are more careful observations on many rays and in many seas, with the use of a camera.

Now we must turn to certain alien but still interesting aids to parturition in viviparous Rays.

How Parturition can be Hastened in the Pregnant Ray

It is now in order to set forth certain external means by which parturition can be forwarded in rays, and particularly to list certain means by which man may effectually hasten the process.

By the Mother Leaping Out of the Sea

First of all, it is alleged that the pregnant females, by leaping, can by the jerk on leaving the water expel the ripe foetus. Most of the large rays are leapers. This is especially true of the Mantids or Devilfishes. These fishes frequent the surface of the sea and are given to leaping and somersaulting. Hill (1863, p. 175) quotes the Jamaican fishermen of his day that the mother *Mobula* leaps to eject the ripe foetus and that the young fish has been observed to fall from her. The southern Florida fishermen know that the rays leap and believe that the young are ejected. I have seen large spotted eagle rays (*Aetobatus narinari*) leap at the Marquesas Atoll, 20 miles west of Key West. It looked as if something fell from some of them, but I was never close enough to be sure.

To me this aid to parturition seems not impossible, and even not improbable. However, if it occurs it would seem that it must be practically confined to rays having large and heavy foetuses, particularly in the Mantids, in which the foetuses by their relatively great weight, if somewhat loose (free) in the uterus, would so react to the leap and to the jerk of the mother's body. Recall here Beebe and Tee-Van's 28-lb. baby *Manta* (p. 79 herein). What is needed is assured evidence—that of the camera above all.

This conjecture, however, must be considered in the light of the well-known fact that the Mantid rays, especially the largest, are much infested with sucking fishes—Remoras. There is a widespread belief (but no actual proof) that the Mantas leap to shake off these commensals. For an excellent account of the infestation of Mantas by these remoras, see Beebe and Tee-Van (1941, p. 275). Whether or not the Sting Rays and Eagle Rays are likewise infested cannot be said in the absence of direct evidence, but it would seem not improbable.

Pain as an Adjuvant to Parturition

Suspending the Ray.—As will now be shown, any pain inflicted on the mother may by reflex action put the uterine muscles into contraction and thus hasten parturition. As early as 1799, Galard de Terraube wrote of a female ray on the coast of French Guiana that, when suspended by a hook over the water, as a result in part of this painful treatment, she let fall into the water a ripe foetus and then three more.

By Unspecified Wounds Inflicted.—The expulsion of the foetus may be facilitated by wounds inflicted by man. Witness Mitchell (1828, p. 28) who after describing a gigantic *Cephalopterus* (*Manta* ?) taken off the mouth of Delaware Bay, U. S. A., states that "... another there, after having been wounded, brought forth in her agony a living young one."

By Piercing Instruments.—This was first noted in 1839. Robert H. Schomburgk, in the introduction to his "Fishes of British Guiana," touches on this subject. It seems that the Indians of the region value rays as food, and in shallow water "flush" then with "pointed poles" and, being very adroit, would transfix the fleeing rays with the poles. Then he adds (1843, II, p. 178) this pertinent statement: "I have frequently observed that the rays, no doubt in consequence of the anguish when secured and transfixed by the poles, brought forth young ones."

At Beaufort, N. C., in the early 1900's (1902–1911) the fishermen used to transfix rays in their nets or in their boats with beardless pikes and transfer them to the laboratory boat. If the rays were advanced in pregnancy, this treatment used often to induce birth.

The following observations of the present writer on pain bringing about premature parturition in rays were made at Beaufort, N. C., in 1909, 1910, and 1911 and were published in 1910 and 1912.

Blows on the Head.—*Rhinoptera bonasus*, the cownosed ray (Fig. 15), in 1909 gave the following interesting observations (Gudger, 1910, p. 397). A gravid female, 24 in. wide, was clubbed on the head to quiet her (in fear of her sting). then, while her abdomen was being opened, she gave birth to two young each 13.5 in. wide and 8.5 in. long (to the end of the ventral fins). Each foetus was rolled up like a sheet of paper—one edge in and one out. This delivery was brought about by muscular contractions of the uterine muscles due to the pain resulting from the clubbing on the head and the slitting of the abdominal wall. Such clubbing sometimes caused death and if the ray was pregnant always resulted in parturition.

Spawn-taker's Technique.—The present writer (Gudger, 1910, p. 399) has had this unusual, perhaps unique, experience with pregnant females of the form *Rhinoptera bonasus*. In "ripe" females, the young slip out very readily through the genital orifice, and on several occasions (July 29, 1902, for the first time) I have delivered the mother of her young by manipulating her abdomen in the manner familiar to spawn-takers.

There have been brought together herein and expanded in orderly format the notes made and laid away at various times during 40 or more years. In itself it seems to be a good deal, but in comparison with the various kinds of sharks and rays in the ocean, it is small, pitifully small. To begin with, we do not know how many of these sharks and rays are viviparous—and, if viviparous, much less do we know anything of their various methods of parturition. The excellent paper of Setna and Sarangdhar is the most extensive known to the writer. More like it are great desiderata. The field is so large and the workers so few.

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THE SEASONAL SUCCESSION OF ORTHOPTERAN STRIDULATION NEAR RALEIGH, NORTH CAROLINA*

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Two Figures

The singing Orthoptera belong mainly in two families, the Tettigoniidae and the Gryllidae. The members of the first family are commonly known as katydids or long-horned grasshoppers, while those of the second family are called crickets. These two families are mainly responsible for the insect chorus so familiarly associated with the late summer and fall, and are the only ones considered in the present study. A third family, the Acrididae, or short-horned grasshoppers, includes a number of species which produce a clattering sound during flight or make a faint rasping sound by rubbing the hind femora against the wings.

The songs of the katydids and crickets are produced by a file and scraper mechanism located near the base of the first pair of wings, a structure which is present only in the male sex. Structures of a different type but apparently having a sound-producing function have been found in the female sex in all local Tettigoniidae except *Atlanticus*. The sounds produced by the females of only a few large species have actually been heard by human ears. They are produced in response to the male song and are weak, brief, and infrequent. The katydids produce rasping or buzzing noises while the crickets generally produce sounds of more musical quality. Most of the songs in both families have some distinctive attributes so that anyone familiar with them can identify the species singing, just as an ornithologist can identify birds by their songs. Some closely related species or subspecies, however, have songs so similar that positive identification from the song alone is not possible. In some cases the habitat or the season of the year may serve as an additional clue to the identity of the species. A few species have more than one type of song which can be produced by the same individual insect. Song types having racial significance, however, occur in *Gryllus* and *Anaxipha* and in such cases each song type is listed separately even though there are no recognized morphological characteristics for separating them into subspecies.

In the vicinity of Raleigh, N. C., it is possible to hear some kind of orthopteran stridulation during ten months of the year, from March to December. Many species have a song period lasting only about two months, while some of the multiple-brooded species may be heard over a continuous period of six months. The seasonal succession starts in March with two species which may be heard during warm nights. The maximum number of species singing is reached in the latter part of August. The number of singers remains high through October,

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but drops off rapidly during November. The number of species recorded as singing during some part of each month is shown in the following table:

	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Tettigoniidae	1	2	2	6	16	21	18	15	3	0
Gryllidae	1	2	5	10	18	26	24	22	13	6
Total	2	4	7	16	34	47	42	37	16	6

In northern states, nearly all of the singing Orthoptera mature in late summer, mostly about the first of August, and continue to sing until the first heavy frost. Many of these species also occur in North Carolina but not all of them follow the same seasonal pattern. Some mature at about the same time or possibly even a little later than they do in the north. Others mature in early summer and are followed by a second brood, or they are single-brooded and die during late summer or early fall. Heavy frosts during October may kill many singing Orthoptera in North Carolina but during some years the weather remains warm and most species die of old age or other natural causes before killing frosts occur. Two species hibernate as adults but do not sing until spring.

The seasonal succession in the stridulation of the Tettigoniidae and Gryllidae is presented in graphical form in Figures 1 and 2. The graphs are based on data compiled from twenty-two years of notes. Most of these notes have been casual lists, jotted down in the field, of species heard singing from time to time. During some years, however, a list of the common species was prepared and checked at fairly regular intervals. Since most of the species sing after dark, most of the observations were made in the evening around the writer's home or while riding in an automobile. The species represented in the graphs are nearly all of the singing Orthoptera known to occur in the Raleigh area. The list does not include a few species that are rare or very restricted in habitat and a few that do not have an audible song.

In the graphs a heavy bar is used to indicate the period between the first and last records. Marks along the top of the bar indicate the dates on which the species was heard singing. These data have been supplemented by date records from adult specimens, indicated by marks below the bar. Where two bars occur on the same line, it means that the species has two well defined singing periods, and that this is a matter of observation and not merely due to a lack of records. In some cases the bar is extended on the basis of specimen records only. This has been done only when the writer believes that the song period probably extends that far. In other cases, a question mark is placed at one end of the bar to indicate that it probably should extend farther, but that the records are incomplete or there is a question of the identity of the species. For species whose song period is not very accurately determined the heavy bar is omitted, and the song and specimen records are spotted in above and below the narrow line.

The beginning of the song period is probably more accurately determined than the end of the period. The observer notices a new song more readily than those

he has become accustomed to hearing. Young males usually sing more than older ones, while toward the end of the song period the number of singers is gradually reduced as the males die off. Since the records were taken during many

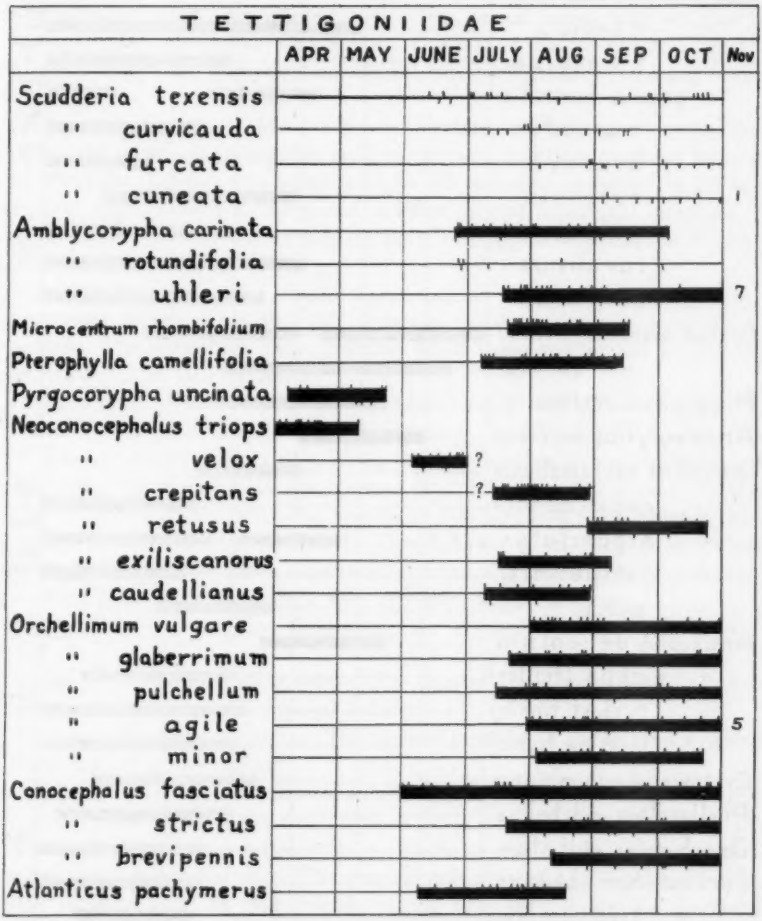

Fig. 1. Song periods of the common long-horned grasshoppers or katydids in the vicinity of Raleigh. See text for explanations.

years and include both early and late seasons, it is possible that the periods indicated may be somewhat longer than the period for a single year. Also, a gap between broods may be shorter than that found in a single year.

Descriptions of the songs have been omitted. Many of the songs have been described in the work of Blatchley (1920) which contains an excellent bibli-

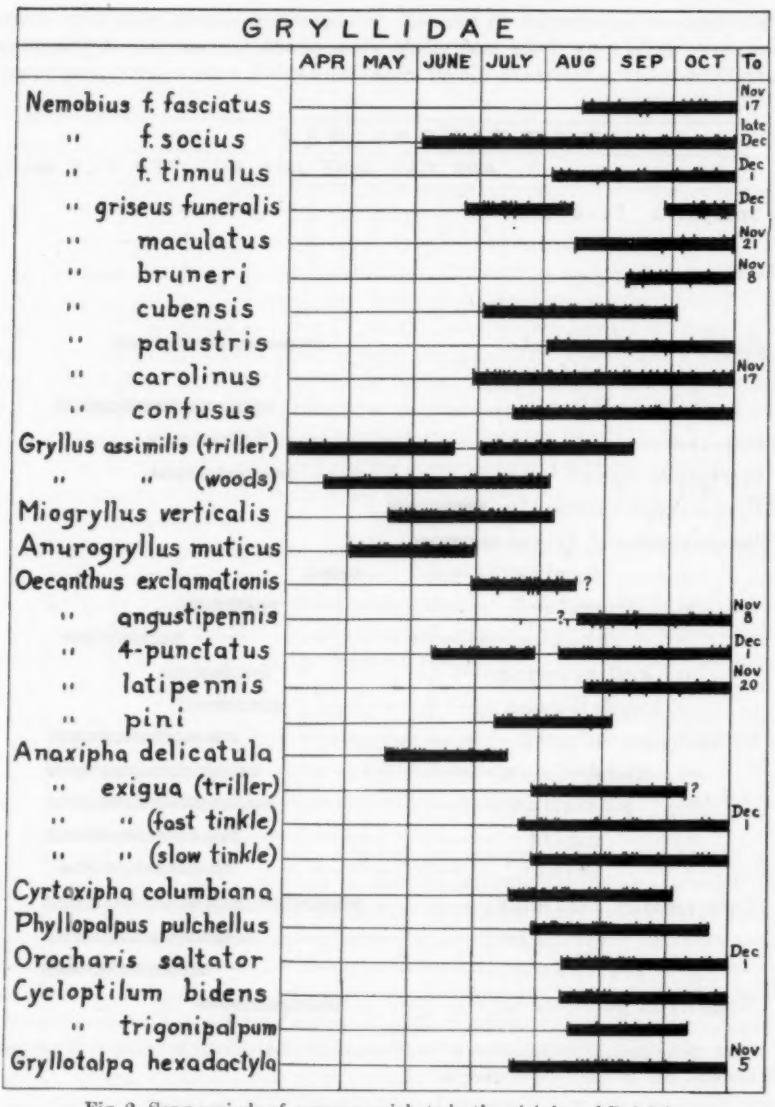

Fig. 2. Song periods of common crickets in the vicinity of Raleigh.

ography of publications up to 1920. Other song descriptions may be found in papers by Snodgrass (1925), Allard (1929), Cantrall (1943) and Fulton (1932). The last publication contains a key to the songs of most of the species mentioned in the present paper.

TETTIGONIIDAE

Scudderia texensis Saussure and Pictet. This species has one type of song that is not like any made by the other species of the genus. It is also less arboreal, frequenting tall grasses and weeds, so that specimens can usually be captured for verification. Adults are present over a long period which may indicate that it has more than one generation per year. The lack of records in late August and early September is probably just accidental, but could possibly be a gap between broods.

S. curvicauda (De Geer). Most of North Carolina is in an area of intergradation between the northern geographic race, *curvicauda*, and the southern race, *laticauda* Brunner. Raleigh specimens seem to the writer to be more like the latter race. Like other species of the genus, it sings only at occasional intervals. The song consists of a single short rasping note, or a rapid repetition of this note, three or four times.

S. furcata Brunner.

S. cuneata Morse. This and the preceding species sing very much like *S. curvicauda*. The collection dates indicate when these species are in the adult stage and probably can be heard.

Amblycorypha floridana carinata Rehn and Hebard. The status of the races of *floridana* is uncertain. Blatchley maintains they are all races of *A. oblongifolia* which has a northern distribution. Along the salt marshes on the North Carolina coast a form occurs with a distinct type of song, which may be typical *floridana*.

A. rotundifolia (Scudder). This species is not common near Raleigh, which accounts for the scarcity of records. Descriptions of its song from different localities do not always agree. Apparently it has a number of local dialects.

A. uhleri Stal. An insect with a very specialized type of song.

Microcentrum rhombifolium (Saussure). An arboreal species with two types of song.

Pterophylla camellifolia (Fabricius). This species is the true katydid, which is seldom seen because of its arboreal habits.

Pyrgocorypha uncinata (Harris). This species matures in the fall but sings only on warm nights in the spring.

Neoconocephalus. The first four members of this genus, *N. triops*, *velox*, *crepitans*, and *retusus* all have the same type of song, a loud continuous buzz. They divide the singing season between them. There seems to be a gap between the song periods of *triops* and *velox*. The song periods of *velox* and *crepitans* probably overlap in early July, so that records during that time should be verified by capture of specimens. The song of *crepitans* probably is louder but the difference is not great enough for identification as it is between *crepitans* and the weak-voiced *retusus*. *N. exiliscanorus* and *caudellianus* sing with rhythmically repeated synchronized notes and can be readily identified by the song.

N. triops (Linnaeus). This species has a seasonal history like *Pyrgocorypha*. It matures about the same time as *N. retusus* but remains silent until spring. It is the only species of katydid observed singing in March.

N. velox Rehn and Hebard. This species was considered rare until the writer discovered its June singing period.

N. robustus crepitans (Scudder).

N. retusus (Scudder).

N. exiliscanorus (Davis).

N. caudellianus (Davis).

Orchelimum. The songs of all members of this genus display some specific features, *glaberrimum* and *minor* being very distinct, while the other three follow a similar pattern.

O. vulgare Harris.

O. glaberrimum (Burmeister).

O. pulchellum Davis. This species is listed as *O. laticauda* Rehn and Hebard by Brimley (1938).

O. agile (De Geer).

O. minor Bruner.

Conocephalus. The members of this genus are very weak singers and can seldom be heard when other insects are singing.

C. fasciatus (De Geer). This species probably has more than one generation in North Carolina.

C. strictus (Scudder).

C. brevipennis (Scudder).

Atlanticus pachymerus (Burmeister). The abbreviated tegmina of this insect appear to be used only for stridulation.

GRYLLIDAE

Nemobius. All members of this genus have characteristic songs except the subspecies of *cubensis* as noted below.

N. fasciatus fasciatus (De Geer). This subspecies, which has only one generation per year, probably matures somewhat earlier in August than indicated by the graph. It can be heard in the Raleigh area only in certain restricted habitats.

N. fasciatus socius Scudder. This race has two and possibly three overlapping generations per year. It can be heard, day or night, for a continuous period from early June until late November and during some years can be heard on warm days until late December. This is the longest continuous song period of any local orthopteran. It is extremely abundant and has the widest habitat range of any member of the genus.

N. fasciatus tinnulus Fulton. A woodland race having one generation per year.

N. griseus funeralis Hart. This southern geographical race, once regarded as extremely rare, can be heard singing in almost any broomsedge (*Andropogon*) field, in midsummer or fall. It has not been recorded in late August or early September although an effort has been made to hear it during that time. Apparently the adults occur in two widely separated broods.

N. maculatus Blatchley. A species found in the climax forest or forest approaching that condition. The scarcity of records is due to the restricted habitat.

N. bruneri Hebard. This cricket is restricted to stream margins. According to the records its song period starts later than any other orthopteran.

N. cubensis cubensis Saussure. This race, which lives among marsh grasses, has a song indistinguishable from that of the *Sphagnum*-inhabiting race, *palustris*.

N. cubensis palustris Blatchley. This subspecies is listed as a species by Brimley (1938). It has been reduced to subspecific rank because it intergrades with *cubensis* in some parts of the United States. In North Carolina, the two races can be separated.

N. carolinus Scudder. The long song period of this species suggests the probability that it has more than one generation but no rearing has been done to prove it.

N. confusus Blatchley.

Gryllus assimilis Fabricius. Two well defined physiological races of the field cricket occur in the Raleigh area. These have not been separated on the basis of visible characters. One race has been called the "triller" because its song is a continuous trill. It lives in fields and has two generations per year. The first generation sings from March to at least the middle of June. This is followed by a period of one or two weeks during which the song disappears or becomes so rare that it has not been observed. In early July the second generation starts singing and continues into September. During some years a few adults sing in late fall. These are early-maturing individuals of the last generation which usually hibernate as large nymphs.

The other race, called "woods" because of its habitat, has only one generation a year. Its song consists of rhythmical chirps like the songs of two other races of *Gryllus* occurring in other parts of North Carolina.

Miogryllus verticalis (Serville).

Anurogryllus muticus (De Geer). Females of this species have been found as late as August 2, a month later than the recorded song period. They carry food to the young nymphs, which remain in the burrow until the second instar.

Oecanthus exclamationis Davis. This species and the following one have songs so similar that they cannot be distinguished with certainty. Both live mainly in the tops of forest trees so that it is difficult to obtain specimens. Probably the song periods overlap. There is no time in August when none can be heard but during some years there has been a noticeable drop in the number singing about the middle of the month.

O. angustipennis Fitch. The earliest adult captured was a recently matured one on July 21, 1930. This is an unusual record in view of the fact that on August 13, 1934, several nymphs were collected but only one adult and on August 25, 1934, some nymphs could still be found.

O. nigricornis quadripunctatus Beutenmuller. This species had two well defined generations per year. In late July or early August the song disappears for a week or two. The singing period of the second generation continues until the crickets are eliminated by heavy frosts.

O. latipennis Riley. The latest-maturing tree cricket. Its song can be identified by its pure tonal quality.

O. pini Beutenmuller. This specie appears to have only one generation per

year, unlike its close relative, *quadripunctatus*. Its song is like that of the latter species but can be identified because it is always in pine trees while the other is in herbaceous vegetation.

Anaxipha delicatula Scudder. This is the species listed by Brimley as *A. pulicaria*. It sings only in spring or early summer, stopping usually before the middle of July. The song is very much like that of the trilling race of *A. exigua* which starts singing about the last of July.

A. exigua (Say). This cricket has three different songs, which seem to differ only in the speed of the wing movements producing them. The race called the "triller" produces a high pitched continuous trill with a very rapid vibrato. It is usually heard in damp woods or shaded marshy places.

The other two forms produce a rhythmical series of distinct tinkling notes, each note apparently made by a single wing stroke. The two songs differ only in the frequency of notes, one being so slow that it is possible to count the notes and the other being a little over twice as rapid. Both tinkling songs are usually heard in the same places, most commonly in dense shrubs and thickets in sunny upland situations.

The distinct ecological distribution of the triller suggests that it may represent a distinct ecological race but the status of the other two is problematical. A few males caged alone were always observed to sing with only one type of song.

The observed periods of the fast and slow tinkle are almost identical. The shorter observed period of the triller may be due to its more restricted distribution as compared to the other two which can be heard about the observer's home.

Cyrtoxipha columbiana Caudell. The only cricket in the area known to synchronize its notes with other males in the same or neighboring trees.

Phyllopalpus pulchellus Uhler. Listed by Brimley as *Phylloscirtus*.

Orocharis saltator Uhler. A few individuals of this species have been recorded in August, but ordinarily the song begins about the first of September.

Cycloptilum bidens Hebard.

C. trigonipalpum (Rehn and Hebard).

Gryllotalpa hexadactyla Perty. The common mole cricket is the only species of mole cricket found in the Raleigh area. Its seasonal history in North Carolina has not been carefully investigated. The song period, extending from late July or August to November, seems clearly established but adult females have been found in April, May, and June, as well as during the song period. The writer's collection and that of the North Carolina Department of Agriculture contain only five male specimens, all of which were taken during the song period. Large nymphs have been found in May, June, July, and September and small ones in July.

An adult female and a large nymph, captured at New Bern, North Carolina, in May, were kept alive in a jar of sand. The adult deposited eggs on May 23 and again in early July. The eggs hatched in about sixteen days, but none of the offspring survived very long. The large nymph matured in July and mated with a male placed in the jar in October.

These data suggest the possibility of a two-year life cycle. According to this

theory the eggs are laid in the spring or early summer; the nymphs mature the following summer; the adults mate during the song period in late summer and fall; the males die during the winter while the females live and deposit eggs the following spring. This would account for the presence of both adults and nymphs at so many different times since there would always be two broods represented.

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ON THE PROJECTIVE-METRIC DEFINITION OF DISTANCE

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In 1853 E. Laguerre (2) showed that the angle θ between two lines l_1 and l_2 may be expressed in projective-metric terms by means of the equation

$$\theta = \frac{1}{2i} \ln (I\, J l_1 l_2) \qquad (1),$$

where I and J are the circular points at infinity.

Cayley (1) in his sixth memoir on quantics effected a generalization of this metric idea by replacing I and J by a proper line conic Γ. Miss Scott (3) provided a critical geometrical appraisal of Cayley's generalization. Cayley's definition of angle expressed analytically is

$$\theta = K. \ln (t_1 t_2 l_1 l_2) \qquad (2),$$

where t_1 and t_2 are the tangents to Γ from P, the vertex of the angle.

Cayley also made a dual definition for distance, using a proper point conic C:

$$d = K. \ln (ABP_1P_2) \qquad (3),$$

where A and B are the points in which the line P_1P_2 meets the conic C.

From Cayley's projective definition of angle one may recover Laguerre's expression by the metric specialization of replacing Γ by the degenerate line conic consisting of I and J as line loci.

However, when one tries to recover the usual expression for distance by replacing Cayley's point conic C by a metric degenerate point conic, and chooses in analogous manner the doubly counted ideal line, he finds that the intersections A and B are coincident, rendering the cross-ratio (ABP_1P_2) unavailable.

We ask now for some conic C which specializes metrically into the twice-counted infinite line, but avoids this duplication of elements. By means of this conic we shall seek through a limit process to obtain the desired expression for distance.

Consider the isotropics on the origin

$$x^2 + y^2 = 0 \qquad (4)$$

together with the ideal line, doubly counted

$$z^2 = 0 \qquad (5).$$

Any linear combination

$$x^2 + y^2 - r^2 z^2 = 0 \qquad (6)$$

of these two conics gives a conic on I and J. These are circles with center at the

origin, one for each radius r. We shall employ circles of this pencil for our conics C, and obtain the line at infinity (twice counted) as the limit of our conics C as $r \to \infty$.

We proceed now to the actual determination of the distance formula, and shall show in detail that

$$d = \lim_{r \to \infty} \frac{r}{2} \ln (ABP_1P_2) \quad \ldots\ldots (7),$$

where r is the radius of the circle (6) and A and B are the points in which the line P_1P_2 meets that circle.

We shall first set the problem up analytically. Typical points on the line P_1P are given parametically by

$$x = \frac{\lambda x_1 + \mu x_2}{\lambda + \mu}, \qquad y = \frac{\lambda y_1 + \mu y_2}{\lambda + \mu} \quad \ldots\ldots (8),$$

where

$$\frac{\mu}{\lambda} = \frac{P_1P}{PP_2}.$$

The points A and B in which the line P_1P_2 meets the circle (6) are given by (8), provided λ and μ satisfy the quadratic equation

$$a\lambda^2 - 2b\lambda\mu + c\mu^2 = 0 \quad \ldots\ldots (9),$$

where

$$\begin{aligned} a &= x_1^2 + y_1^2 - r^2 \\ -b &= x_1x_2 + y_1y_2 - r^2 \\ c &= x_2^2 + y_2^2 - r^2. \end{aligned}$$

The discriminant D of (9), needed in the sequel, has the value

$$D = (x_1x_2 + y_1y_2 - r^2)^2 - (x_1^2 + y_1^2 - r^2)(x_2^2 + y_2^2 - r^2) \quad \ldots\ldots (10),$$

which simplifies into

$$D = \{(x_1 - x_2)^2 + (y_1 - y_2)^2\}r^2 - (x_1y_2 - x_2y_1)^2 \quad \ldots\ldots (11).$$

We are now in position to express the cross-ratio analytically. We have

$$(A\,B\,P_1\,P_2) = \frac{AP_1}{BP_1}\Big/\frac{AP_2}{BP_2} = \frac{P_1A}{AP_2}\Big/\frac{P_1B}{BP_2} = \frac{\mu_1}{\lambda_1}\Big/\frac{\mu_2}{\lambda_2} \quad \ldots\ldots (12),$$

where the two values of $\frac{\mu}{\lambda}$ are solutions of (9). Consequently, we obtain

$$(A\,B\,P_1\,P_2) = \frac{b + \sqrt{D}}{c}\Big/\frac{b - \sqrt{D}}{c} = \frac{b + \sqrt{D}}{b - \sqrt{D}} = \frac{1 + x}{1 - x} \quad \ldots\ldots (13),$$

where $x = \dfrac{\sqrt{D}}{b}$.

Now

$$x^2 = \frac{D}{b^2} = \frac{\{(x_1 - x_2)^2 + (y_1 - y_2)^2\}\, r^2 - (x_1 y_2 - x_2 y_1)^2}{r^4 - 2(x_1 x_2 + y_1 y_2) r^2 + (x_1 x_2 + y_1 y_2)^2} \quad \ldots\ldots\ldots\ldots (14),$$

where $x^2 \to 0$ as $r \to \infty$, and therefore $x^2 < 1$ for r sufficiently large.

Now by (13)

$$\ln (A B P_1 P_2) = \ln \frac{1 + x}{1 - x} = 2\left[x + \frac{x^3}{3} + \frac{x^5}{5} + \cdots .\right] \quad \ldots\ldots\ldots\ldots (15),$$

a convergent series since $x^2 < 1$, (Peirce 769).

From (15) we may write

$$\frac{1}{2} \ln (A B P_1 P_2) = x \left\{1 + \frac{x}{3} + \frac{x^4}{5} + \cdots .\right\} \quad \ldots\ldots\ldots\ldots (16).$$

But from (14)

$$r^2 x^2 \to (x_1 - x_2)^2 + (y_1 - y_2)^2 \quad \ldots\ldots\ldots\ldots (17)$$

as $r \to \infty$ and $x \to 0$, consequently from (16)

$$\lim \frac{r}{2} \ln (A B P_1 P_2) = \lim rx \cdot \lim \left\{1 + \frac{x}{3} + \frac{x^4}{5} + \cdots .\right\} \quad \ldots\ldots\ldots\ldots (18),$$

and finally

$$\lim_{r \to \infty} \frac{r}{2} \ln (A B P_1 P_2) = d \quad \ldots\ldots\ldots\ldots (19),$$

where

$$d = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2} \quad \ldots\ldots\ldots\ldots (20),$$

the usual formula for the distance between two points.

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SOME THEOREMS ON THE APPROXIMATION OF IRRATIONAL NUMBERS BY THE CONVERGENTS OF THEIR CONTINUED FRACTIONS[1]

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1. *Introduction.* Let ξ be any positive irrational number. We shall consider the expansion of ξ as the regular continued fraction

$$\xi = [q_0, q_1, q_2, \cdots].$$

Let us denote the numerators and denominators of its convergents by A_n and B_n, respectively, $(n = 0, 1, 2, \cdots)$, and set

$$|\xi - A_n/B_n| = 1/(\lambda_n B_n^2).$$

Hurwitz [4] proved that for any given ξ there exist infinitely many $\lambda_n > \sqrt{5}$, and that there exist numbers ξ for which only a finite number of the λ_n are greater than c for any $c > \sqrt{5}$. Vahlen (5) showed that for every ξ at least one of two consecutive λ_n exceeds 2. Borel (1) proved that for every ξ and every n we have $\max(\lambda_n, \lambda_{n+1}, \lambda_{n+2}) > \sqrt{5}$.

In two recent papers, Brauer and Macon (2, 3) obtained extensions of these results. Hereafter, these papers will be referred to as *BM-I* and *BM-II* respectively.

In *BM-II*, best possible lower bounds were obtained for the sums of arbitrary numbers of consecutive λ_i. For example, it was proved that for every ξ and every n we have $\lambda_n + \lambda_{n+1} > 4$, $\lambda_n + \lambda_{n+1} + \lambda_{n+2} > 6$, $\lambda_n + \lambda_{n+1} + \lambda_{n+2} + \lambda_{n+3} > 25/3$, and that these bounds cannot be improved.

Instead of the sums of consecutive λ_i, the products of these numbers will be considered here. In *BM-I* the inequality $\lambda_n\lambda_{n+1} > 4$ was obtained. It will be proved here that this result cannot be improved farther. Moreover, it will be shown that $\lambda_n\lambda_{n+1}\lambda_{n+2} > 27/4$ and $\lambda_n\lambda_{n+1}\lambda_{n+2}\lambda_{n+3} > 625/36$ for every $n \geqq 0$. These results will be shown to be the best possible. Just as the bounds for the sums of three and four consecutive λ_i place certain restrictions on the domains of values of the λ_i in 3-space and 4-space, respectively, these bounds for the products impose additional restrictions on the domains of values of the λ_i.

Furthermore, it would follow from Borel's Theorem only that at least one of five consecutive λ_i exceeds $\sqrt{5}$. In *BM-I*, the following theorems were proved:

For every irrational number ξ, either at least two of five consecutive λ_i exceed $\sqrt{5}$, or at least one of them exceeds 3.

[1] This is an abridged form of a thesis submitted to the Faculty of the University of North Carolina in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Department of Mathematics. The author wishes to express his appreciation for the guidance of Professor Alfred T. Brauer in the preparation of this paper.

Let t be any number of form $3m + 2$. In each sequence of t consecutive λ_i there are either at least $m + 1$ elements which exceed $\sqrt{5}$ or m elements which exceed 3.

The result for five consecutive λ_i cannot be improved in general. However, from these results one could only say of each sequence of eight consecutive λ_i either that at least three exceed $\sqrt{5}$ or that two exceed 3. It will be proved here that if in any sequence of eight consecutive λ_i only two λ_i exceed $\sqrt{5}$, then either both of them exceed $3 + 3/38$ or one exceeds $4 + 3/38$. A similar result for eleven consecutive λ_i will be obtained.

2. *The products of two, three, and four consecutive* λ_i. In *BM-I*, Theorem 4, it was shown that $\lambda_n\lambda_{n+1} > 4$, $(n = 0, 1, 2, \cdots)$. We shall first show that this result cannot be improved farther.

Consider the set of continued fractions for which $q_{n+1} = q_{n+2} = 1$. It follows from (3), (6), and (7) of *BM-I*, that

$$\lambda_n = \xi_{n+1} + \phi_{n+1} = [1, 1, \xi_{n+3}] + [0, q_n + \phi_n] < [1, 1, q_{n+3} + 1] + [0, q_n] = 1 + (q_{n+3} + 1)/(q_{n+3} + 2) + 1/q_n,$$

since $\phi_n \leqq 1$. Moreover,

$$\lambda_{n+1} = \xi_{n+2} + \phi_{n+2} = [1, \xi_{n+3}] + [0, 1, q_n + \phi_n] < [1, q_{n+3}] + [0, 1, q_n + 1] = 1 + 1/q_{n+3} + (q_n + 1)/(q_n + 2).$$

Hence, for continued fractions of this set, we have

$$\lambda_n\lambda_{n+1} < [1 + (q_{n+3} + 1)/(q_{n+3} + 2) + 1/q_n]\cdot[1 + 1/q_{n+3} + (q_n + 1)/(q_n + 2)].$$

Since q_n and q_{n+3} are variables, given $\epsilon > 0$ there exists an N such that $q_n > N$ and $q_{n+3} > N$ imply $\lambda_n\lambda_{n+1} < 4 + \epsilon$ for the number

$$\xi = [q_0, q_1, \cdots, q_n, 1, 1, q_{n+3}, \cdots].$$

THEOREM I. For every $\epsilon > 0$, it is possible to construct irrational numbers ξ for which

$$\lambda_n\lambda_{n+1} < 4 + \epsilon$$

for infinitely many values of n.

BM-I, Theorem 4 and (20), can be extended to three consecutive λ_i as follows:

THEOREM II. The inequality

$$\lambda_n\lambda_{n+1}\lambda_{n+2} > 27/4$$

holds for all irrational numbers ξ and all n. This is the best possible lower bound.

PROOF: It follows from *BM-I*, Theorem 4, that

$$\lambda_n\lambda_{n+1}\lambda_{n+2} > \lambda_n(\lambda_{n+1} + \lambda_{n+2}) = \lambda_n\lambda_{n+1} + \lambda_n\lambda_{n+2} > \lambda_n + \lambda_{n+1} + \lambda_n\lambda_{n+2},$$

and from *BM-I*, Theorem 5, that

$$\lambda_n + \lambda_{n+1} + \lambda_n\lambda_{n+2} > \lambda_{n+1}/(\lambda_{n+1} - 1) + \lambda_{n+1} + \lambda_{n+1}^2/(\lambda_{n+1} - 1)^2 = \lambda_{n+1}^3/(\lambda_{n+1} - 1)^2.$$

Hence we have

$$\lambda_n\lambda_{n+1}\lambda_{n+2} > \lambda_{n+1}^3/(\lambda_{n+1} - 1)^2. \tag{1}$$

Let us write λ for λ_{n+1}. The maxima and minima for the right hand side of (1) are given by the real roots of

$$3\lambda^2(\lambda^2 - 2\lambda + 1) - \lambda^3(2\lambda - 2) = 0;$$

that is,

$$\lambda^2(\lambda^2 - 4\lambda + 3) = 0.$$

Thus, for $\lambda > 1$, this function assumes its only minimum at $\lambda = 3$. Hence,

$$\lambda_n\lambda_{n+1}\lambda_{n+2} > \lambda_{n+1}^3/(\lambda_{n+1} - 1)^2 \geqq 3^3/(3 - 1)^2 = 27/4.$$

To prove the second part, consider the continued fractions of the form

$$[q_0, q_1, \cdots, q_n, 1, 1, 1, q_{n+4}, \cdots].$$

For these numbers, it follows from *BM-I*, (6) and (7), that:

$$\xi_{n+1} = [1, 1, 1, \xi_{n+4}] = 1 + (\xi_{n+4} + 1)/(2\xi_{n+4} + 1), \tag{2}$$

$$\xi_{n+2} = [1, 1, \xi_{n+4}] = 1 + \xi_{n+4}/(\xi_{n+4} + 1), \tag{3}$$

$$\xi_{n+3} = [1, \xi_{n+4}] = 1 + 1/\xi_{n+4}, \tag{4}$$

$$\phi_{n+1} = [0, q_n + \phi_n] = 1/(q_n + \phi_n), \tag{5}$$

$$\phi_{n+2} = [0, 1, q_n + \phi_n] = (q_n + \phi_n)/(q_n + \phi_n + 1), \tag{6}$$

$$\phi_{n+3} = [0, 1, 1, q_n + \phi_n] = (q_n + \phi_n + 1)/[2(q_n + \phi_n) + 1]. \tag{7}$$

Since $\xi_{n+4} = q_{n+4} + 1/\xi_{n+5}$, we have from (2), (3), and (4), as $q_{n+4} \to \infty$: $\lim \xi_{n+1} = 3/2$, $\lim \xi_{n+2} = 2$, and $\lim \xi_{n+3} = 1$. Similarly, since $0 \leqq \phi_n \leqq 1$, we obtain from (5), (6), and (7), as $q_n \to \infty$; $\lim \phi_{n+1} = 0$, $\lim \phi_{n+2} = 1$, and $\lim \phi_{n+3} = 1/2$. Thus it follows from *BM-I*, (3), that if $q_n \to \infty$ and $q_{n+4} \to \infty$, then $\lim \lambda_n = 3/2$, $\lim \lambda_{n+1} = 3$, and $\lim \lambda_{n+2} = 3/2$. Thus, we have $\lim \lambda_n\lambda_{n+1}\lambda_{n+2} = 27/4$.

Let us consider four consecutive λ_i. We need the following lemma:

LEMMA I. If at least one of q_{n+1}, q_{n+2}, q_{n+3}, and q_{n+4} is greater than 2, then $\lambda_n\lambda_{n+1}\lambda_{n+2}\lambda_{n+3} > 18$.

PROOF. Four cases arise.

Case 1. $q_{n+2} \geqq 2$.

It follows from *BM-I*, (7), that $\phi_{n+2} = 1/(q_{n+1} + \phi_{n+1}) < 1/2$. Thus, by *BM-I*, Theorem 4 and (3),

$$\lambda_n\lambda_{n+1} > \lambda_n + \lambda_{n+1} = 1/\xi_{n+2} + 1/\phi_{n+2} + \xi_{n+2} + \phi_{n+2} \geqq 1/\phi_{n+2} + \phi_{n+2} + 2,$$

since, for $x > 0$, the function $x + 1/x$ assumes its only minimum at $x = 1$. But, since $0 < \phi_{n+2} < 1/2$, we have $1/\phi_{n+2} + \phi_{n+2} > 5/2$. Hence, $\lambda_n\lambda_{n+1} > 9/2$; and, since $\lambda_{n+2}\lambda_{n+3} > 4$, we have $\lambda_n\lambda_{n+1}\lambda_{n+2}\lambda_{n+3} > 18$.

Case 2. $q_{n+2} \geqq 2$.

It follows from *BM-I*, (6), that $\xi_{n+2} = q_{n+2} + 1/\xi_{n+3} > 2$. Hence

$$\lambda_n\lambda_{n+1} > 1/\phi_{n+2} + \phi_{n+2} + 1/\xi_{n+2} + \xi_{n+2} > 2 + 5/2 = 9/2.$$

But again $\lambda_{n+2}\lambda_{n+3} > 4$.

Case 3. $q_{n+3} \geqq 2$.

Similarly to Case 1, it follows here that $\phi_{n+4} < 1/2$, and thus $\lambda_{n+2}\lambda_{n+3} > 9/2$. Since $\lambda_n\lambda_{n+1} > 4$, we have $\lambda_n\lambda_{n+1}\lambda_{n+2}\lambda_{n+3} > 18$.

Case 4. $q_{n+4} \geqq 2$.

Similarly to Case 2, we have $\xi_{n+4} > 2$, and hence $\lambda_{n+2}\lambda_{n+3} > 9/2$. Thus the proof is complete.

THEOREM III. The inequality

$$\lambda_n\lambda_{n+1}\lambda_{n+2}\lambda_{n+3} > 625/36$$

holds for all irrational numbers ξ and all n. This is the best possible lower bound.

PROOF. It follows from the lemma that we need to consider only the case in which $q_{n+i} = 1$, $(i = 1, 2, 3, 4)$. In this case, the following relations follow from *BM-I*, (6):

$$\begin{aligned}
\xi_{n+4} &= q_{n+4} + 1/\xi_{n+5} = 1 + 1/\xi_{n+5} \\
\xi_{n+3} &= q_{n+3} + 1/\xi_{n+4} = 1 + \xi_{n+5}/(\xi_{n+5} + 1) \\
\xi_{n+2} &= q_{n+2} + 1/\xi_{n+3} = 1 + (\xi_{n+5} + 1)/(2\,\xi_{n+5} + 1) \\
\xi_{n+1} &= q_{n+1} + 1/\xi_{n+2} = 1 + (2\xi_{n+5} + 1)/(3\,\xi_{n+5} + 2).
\end{aligned}$$

Similarly, it follows from *BM-I*, (7), that

$$\begin{aligned}
\phi_{n+1} &= 1/(q_n + \phi_n) \\
\phi_{n+2} &= (q_n + \phi_n)/[(q_n + \phi_n) + 1] \\
\phi_{n+3} &= [(q_n + \phi_n) + 1]/[2(q_n + \phi_n) + 1] \\
\phi_{n+4} &= [2(q_n + \phi_n) + 1]/[3(q_n + \phi_n) + 2].
\end{aligned}$$

For simplicity, let us write $x = \xi_{n+5}$ and $y = q_n + \phi_n$. Then we have from *BM-I*, (3), that

$$(8)\quad \begin{cases}
\lambda_n = 1 + (2x+1)/(3x+2) + 1/y = (2x+1)/(3x+2) + (y+1)/y, \\
\lambda_{n+1} = 1 + (x+1)/(2x+1) + y/(y+1) \\
\qquad\qquad = (3x+2)/(2x+1) + y/(y+1), \\
\lambda_{n+2} = 1 + x/(x+1) + (y+1)/(2y+1) \\
\qquad\qquad = x/(x+1) + (3y+2)/(2y+1), \\
\lambda_{n+3} = 1 + 1/x + (2y+1)/(3y+2) = (x+1)/x + (2y+1)/(3y+2).
\end{cases}$$

Hence,

$$(9)\quad \begin{aligned}
\lambda_n\lambda_{n+1} &= \left(\frac{2x+1}{3x+2} + \frac{y+1}{y}\right)\left(\frac{3x+2}{2x+1} + \frac{y}{y+1}\right) \\
&= 2 + \frac{2x+1}{3x+2}\left(\frac{y}{y+1}\right) + \frac{3x+2}{2x+1}\left(\frac{y+1}{y}\right).
\end{aligned}$$

Thus, for $x > 0$ and $y > 0$,

$$\frac{\partial}{\partial x}(\lambda_n\lambda_{n+1}) = \frac{1}{(3x+2)^2}\cdot\frac{y}{y+1} - \frac{1}{(2x+1)^2}\cdot\frac{y+1}{y} < 0,$$

since $(3x+2)^{-2} < (2x+1)^{-2}$ and $y/(y+1) < (y+1)/y$. Therefore $\lambda_n\lambda_{n+1}$ is

a decreasing function of x for fixed y; and so

$$\lambda_n\lambda_{n+1} > 2 + 2y/3(y+1) + 3(y+1)/2y. \tag{10}$$

Differentiating the right member of (10) with respect to y, we obtain

$$2/3(y+1)^2 - 3/2y^2,$$

which clearly is negative for $y > 0$. Hence the right member of (10) is a decreasing function of y, and we have

$$\lambda_n\lambda_{n+1} > 2 + 2/3 + 3/2 = 25/6.$$

In a similar manner, we have from (8)

$$\lambda_{n+2}\lambda_{n+3} = \left(\frac{x}{x+1} + \frac{3y+2}{2y+1}\right)\left(\frac{x+1}{x} + \frac{2y+1}{3y+2}\right).$$

It will be noted that the right member of the above is exactly the same as the right member of (9) with x and y interchanged. Thus, in exactly the same way, it follows that $\lambda_{n+2}\lambda_{n+3} > 25/6$. Therefore,

$$\lambda_n\lambda_{n+1}\lambda_{n+2}\lambda_{n+3} > 625/36.$$

Since $x = \xi_{n+3}$ and $y = q_n + \phi_n$ can be made as large as we please by choosing a number

$$\xi = [q_0, q_1, \cdots, q_n, 1, 1, 1, 1, q_{n+5}, \cdots]$$

with sufficiently large q_n and q_{n+5}, it is clear that the bound 625/36 cannot be improved.

3. *Some extensions of Borel's Theorem.* Borel [1] proved that at least one of three consecutive λ_i is greater than $\sqrt{5}$. In *BM-I*, Theorem 9, it was shown that either at least two of five consecutive λ_i exceed $\sqrt{5}$, or at least one is greater than 3. These theorems will now be extended to sequences of eight and eleven consecutive λ_i.

THEOREM IV. If in any sequence of eight consecutive λ_i, say $\lambda_n, \lambda_{n+1}, \cdots, \lambda_{n+7}$, only two elements exceed $\sqrt{5}$, then either both of them exceed $3 + 3/38$ or one exceeds $4 + 3/38$.

PROOF. Since only two λ_i exceed $\sqrt{5}$, then by *BM-I*, Theorem 9, we have $\lambda_{n+2} > 3$ and $\lambda_{n+5} > 3$. Thus it follows from *BM-I*, Theorem 1, that

$$\min(\phi_{n+2}, \phi_{n+5}, \phi_{n+8}) > (\sqrt{5} - 1)/2. \tag{11}$$

Hence, by *BM-I*, (5),

$$\max(\phi_{n+1}, \phi_{n+3}, \phi_{n+4}, \phi_{n+6}, \phi_{n+7}, \phi_{n+9}) < (\sqrt{5} - 1)/2. \tag{12}$$

Thus it follows from *BM-I*, (24), that

$$q_{n+3} \geqq 2 \quad \text{and} \quad q_{n+6} \geqq 2. \tag{13}$$

Moreover, it follows from (11) and *BM-I*, Theorem 2, that

$$q_{n+1} = q_{n+4} = q_{n+7} = 1. \tag{14}$$

Since $\lambda_{n+1} = \xi_{n+2} + \phi_{n+2} < \sqrt{5}$, we have from (11)

$$\xi_{n+2} < \sqrt{5} - \phi_{n+2} < \sqrt{5} - (\sqrt{5} - 1)/2 < 2.$$

Hence, from *BM-I*, (6),

$$q_{n+2} = 1. \tag{15}$$

Similarly,

$$q_{n+5} = q_{n+8} = 1. \tag{16}$$

But it follows from *BM-I*, (3), (6), and (7), that

$$\lambda_{n+2} = [q_{n+3}, q_{n+4}, q_{n+5}, q_{n+6}, q_{n+7}, q_{n+8}, \xi_{n+9}] + [0, q_{n+2}, q_{n+1} + \phi_{n+1}].$$

Consequently, it follows from (13), (14), (15), and (16) that

$$\lambda_{n+2} = [q_{n+3}, 1, 1, q_{n+6}, 1, 1, \xi_{n+9}] + [0, 1, 1 + \phi_{n+1}]. \tag{17}$$

Similarly, we have

$$\lambda_{n+5} = [q_{n+6}, 1, 1, \xi_{n+9}] + [0, 1, 1, q_{n+3}, 1, 1 + \phi_{n+1}]. \tag{18}$$

Four cases arise:

Case 1. $q_{n+3} = q_{n+6} = 2$.

From (17), we have

$$\lambda_{n+2} = [2, 1, 1, 2, 1, 1, \xi_{n+9}] + [0, 1, 1 + \phi_{n+1}] > [2, 1, 1, 2, 1, 1, 1] + [0, 1, 1] = 3 + 3/38.$$

Similarly, from (18) and (12), we have

$$\lambda_{n+5} = [2, 1, 1, \xi_{n+9}] + [0, 1, 1, 2, 1, 1 + \phi_{n+1}] > [2, 1, 1] + [0, 1, 1, 2, 1, 1 + (\sqrt{5} - 1)/2] = 5/2 + (15 - \sqrt{5})/22 > 3 + 3/38.$$

Case 2. $q_{n+3} = 2$, $q_{n+6} \geqq 3$.

It follows from (18) and (12) that

$$\lambda_{n+5} > [3, 1, 1] + [0, 1, 1, 2, 1, 1 + (\sqrt{5} - 1)/2] > 4 + 3/38.$$

Case 3. $q_{n+3} \geqq 3$, $q_{n+6} = 2$.

We have from (17)

$$\lambda_{n+2} > [3, 1, 1, 2, 1, 1, 1] + [0, 1, 1] = 4 + 3/38$$

Case 4. $q_{n+3} \geqq 3$, $q_{n+6} \geqq 3$.

From (17), it follows here that

$$\lambda_{n+2} = [q_{n+3}, 1, 1, \xi_{n+6}] + [0, 1, 1 + \phi_{n+1}] > [3, 1, 1] + [0, 1, 1] = 4.$$

Similarly, from (18),

$$\lambda_{n+5} = [q_{n+6}, 1, 1, \xi_{n+9}] + [0, 1, 1 + \phi_{n+4}] > [3, 1, 1] + [0, 1, 1] = 4.$$

THEOREM V. If in any sequence of eleven consecutive λ_i, say $\lambda_n, \lambda_{n+1}, \cdots, \lambda_{n+10}$, only three λ_i exceed $\sqrt{5}$, then at least one of the following conditions is

satisfied:

(a) one λ_i exceeds $3 + (109 - 19\sqrt{5})/418$, and two exceed $3 + 3/37$;
(b) one λ_i exceeds $4 + 3/38$, and one exceeds 4;
(c) one λ_i exceeds $4 + 3/37$, one exceeds $3 + 7/86$, and one exceeds $3 + 3/38$;
(d) one λ_i exceeds $4 + (109 - 19\sqrt{5})/418$.

PROOF. Since only three λ_i exceed $\sqrt{5}$, it follows from *BM-I*, Theorem 1,

$$\min(\phi_{n+i}) > (\sqrt{5} - 1)/2, \qquad (i = 2, 5, 8, 11); \tag{19}$$

and so, by *BM-I*, (5),

$$\max(\phi_{n+1}) < (\sqrt{5} - 1)/2, \qquad (i = 1, 3, 4, 6, 7, 9, 10, 12). \tag{20}$$

Thus we can prove in exactly the same way as for Theorem IV that

$$q_{n+i} = 1, \qquad i = 1, 2, 4, 5, 7, 8, 10, 11), \tag{21}$$

and that

$$q_{n+i} \geqq 2, \qquad (i = 3, 6, 9). \tag{22}$$

Therefore we have from *BM-I*, (3), (6), and (7), and from (21) that

$$\lambda_{n+2} = [q_{n+3}, 1, 1, q_{n+6}, 1, 1, q_{n+9}, 1, 1, \xi_{n+12}] + [0, 1, 1 + \phi_{n+1}]; \tag{23}$$

$$\lambda_{n+5} = [q_{n+6}, 1, 1, q_{n+9}, 1, 1, \xi_{n+12}] + [0, 1, 1, q_{n+3}, 1, 1 + \phi_{n+1}]; \tag{24}$$

$$\lambda_{n+8} = [q_{n+9}, 1, 1, \xi_{n+12}] + [0, 1, 1, q_{n+6}, 1, 1, q_{n+3}, 1, 1 + \phi_{n+1}]. \tag{25}$$

Eight cases arise:

Case 1. $q_{n+3} = q_{n+6} = q_{n+9} = 2$.

It follows from (23) that

$$\lambda_{n+2} > [2, 1, 1, 2, 1, 1, 2, 1, 1] + [0, 1, 1] = 3 + 3/37.$$

From (24) and (20), we have

$$\lambda_{n+5} > [2, 1, 1, 2, 1, 1, 1] + [0, 1, 1, 2, 1, 1 + (\sqrt{5} - 1)/2] = 3 + (109 - 19\sqrt{5})/418.$$

Finally, it follows from (25) that

$$\lambda_{n+8} > [2, 1, 1] + [0, 1, 1, 2, 1, 1, 2, 1, 1] = 3 + 3/37.$$

Hence (a) is satisfied.

Case 2. $q_{n+3} \geqq 3$, $q_{n+6} = q_{n+9} = 2$.

From (23), we obtain

$$\lambda_{n+2} > [3, 1, 1, 2, 1, 1, 2, 1, 1] + [0, 1, 1] = 4 + 3/37.$$

It follows from (24) that

$$\lambda_{n+5} = [2, 1, 1, 2, 1, 1, \xi_{n+12}] + [0, 1, 1, q_{n+3} + \phi_{n+3}] > [2, 1, 1, 2, 1, 1, 1] + [0, 1, 1] = 3 + 3/38$$

Finally, we have from (25)

$$\lambda_{n+3} = [2, 1, 1, \xi_{n+12}] + [0, 1, 1, 2, 1, 1, q_{n+3} + \phi_{n+3}] > [2, 1, 1] + [0, 1, 1, 2, 1, 1, 3] = 3 + 7/86.$$

Thus (c) is satisfied.

Case 3. $q_{n+3} = 2$, $q_{n+6} \geqq 3$, $q_{n+9} = 2$.

It follows from (24) and (20) that

$$\lambda_{n+5} > [3, 1, 1, 2, 1, 1, 1] + [0, 1, 1, 2, 1, 1 + (\sqrt{5} - 1)/2] = 4 + (109 - 19\sqrt{5})/418.$$

Hence (*d*) is satisfied.

Case 4. $q_{n+3} = q_{n+6} = 2$, $q_{n+9} \geqq 3$.

We have from (23), (24), and (25)

$$\lambda_{n+2} = [2, 1, 1, 2, 1, 1, \xi_{n+9}] + [0, 1, 1 + \phi_{n+1}] > [2, 1, 1, 2, 1, 1, 3] + [0, 1, 1] = 3 + 7/86,$$

$$\lambda_{n+5} = [2, 1, 1, \xi_{n+9}] + [0, 1, 1, 2, 1, 1 + \phi_{n+1}] > [2, 1, 1] + [0, 1, 1, 2, 1, 1 + (\sqrt{5} - 1)/2] = 3 + (4 - \sqrt{5})/22 > 3 + 3/38,$$

$$\lambda_{n+3} > [3, 1, 1] + [0, 1, 1, 2, 1, 1, 2, 1, 1] = 4 + 3/37.$$

Hence (c) is again satisfied.

Case 5. $q_{n+3} \geqq 3$, $q_{n+6} \geqq 3$, $q_{n+9} = 2$.

It follows from (23) and (24) that

$$\lambda_{n+2} = [q_{n+3}, 1, 1, \xi_{n+6}] + [0, 1, 1 + \phi_{n+1}] > [3, 1, 1] + [0, 1, 1] = 4,$$

$$\lambda_{n+5} = [q_{n+6}, 1, 1, 2, 1, 1, \xi_{n+12}] + [0, 1, 1, q_{n+3} + \phi_{n+3}] > [3, 1, 1, 2, 1, 1, 1] + [0, 1, 1] = 4 + 3/38.$$

Thus (b) holds.

Case 6. $q_{n+3} \geqq 3$, $q_{n+6} = 2$, $q_{n+9} \geqq 3$.

From (23) and (25) we have

$$\lambda_{n+2} = [q_{n+3}, 1, 1, 2, 1, 1, \xi_{n+9}] + [0, 1, 1 + \phi_{n+1}] > [3, 1, 1, 2, 1, 1, 3] + [0, 1, 1] = 4 + 7/86 > 4 + 3/38,$$

$$\lambda_{n+5} = [q_{n+9}, 1, 1, \xi_{n+12}] + [0, 1, 1, 2, 1, 1, q_{n+3} + \phi_{n+3}] > [3, 1, 1] + [0, 1, 1, 2, 1, 1, 3] = 4 + 7/86 > 4.$$

Hence (b) is satisfied.

Case 7. $q_{n+3} = 2$, $q_{n+6} \geqq 3$, $q_{n+9} \geqq 3$.

We obtain from (24) and (25)

$$\lambda_{n+5} = [q_{n+6}, 1, 1, \xi_{n+9}] + [0, 1, 1, 2, 1, 1 + \phi_{n+1}] > [3, 1, 1] + [0, 1, 1, 2, 1, 1 + (\sqrt{5} - 1)/2] = 4 + (4 - \sqrt{5})/22 > 4 + 3/38,$$

$$\lambda_{n+3} = [q_{n+9}, 1, 1, \xi_{n+12}] + [0, 1, 1, q_{n+6} + \phi_{n+6}] > [3, 1, 1] + [0, 1, 1] = 4.$$

Therefore (b) also holds here.

Case 8. $q_{n+3} \geqq 3$, $q_{n+6} \geqq 3$, $q_{n+9} \geqq 3$.
From (23), (24), and (25), we have

$$\lambda_{n+2} = [q_{n+3}, 1, 1, \xi_{n+6}] + [0, 1, 1 + \phi_{n+1}] > [3, 1, 1] + [0, 1, 1] = 4,$$
$$\lambda_{n+5} = [q_{n+6}, 1, 1, \xi_{n+9}] + [0, 1, 1 + \phi_{n+4}] > [3, 1, 1] + [0, 1, 1] = 4,$$
$$\lambda_{n+8} = [q_{n+9}, 1, 1, \xi_{n+12}] + [0, 1, 1 + \phi_{n+7}] > [3, 1, 1] + [0, 1, 1] = 4.$$

Hence (a) is satisfied, and the proof is complete.

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A NEW SPECIES OF SORODISCUS ON PYTHIUM*

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Plates 1 and 2

In samples of soil collected in Florida in 1949, a species of *Pythium* was found parasitized by a member of the Plasmodiophoraceae which forms its resting spores characteristically in two-layered discs, as in the genus *Sorodiscus*. Zoosporangia have been seen for the first time in this genus. The three other species, which were described largely from fixed material, occur as parasites of flowering plants and *Chara*. Only two other genera of the Plasmodiophoraceae have been found as parasites of fungi; these are *Octomyxa* and *Woronina*, from which the new organism described below is entirely distinct. The affinity of this organism with the Plasmodiophoraceae, suggested by the formation of the typical heterokont zoospores, is shown conclusively by the occurrence of cruciform divisions of the nuclei during the vegetative growth of the plasmodium. Its life cycle and host relationships necessitate the identification of this organism as a new species.

Sorodiscus Cokeri n. sp.

Fungus parasiticus in hyphis Pythii; non excitans tumores. Maturo plasmodio formante sorum zoosporangiorum vel sporarum perdurantium. Zoosporangiis globosis vel ovoideis, 5.3–8.8μ in diam. Zoosporis biflagellatis, posteriore flagello longo, anteriore brevi, 4–11, plerumque 8 vel 9, in zoosporangio. Sporis perdurantibus 3.1–4.1μ in diam., levibus, cum parietibus incrassatis; sporis ordinatis in discibus, crassitudinis singularium sporarum, 2–4, plerumque 2, in cystosoro, firmiter compactis.

Obligate parasite on species of *Pythium*, in swellings homologous with the sporangia of the host, causing little or no hypertrophy. Naked plasmodium undergoing cruciform nuclear divisions during vegetative growth; at maturity partly or occasionally wholly filling the swelling, and dividing into zoosporangia or resting spores. Zoosporangia thin-walled, spherical to ovoid, 5.3–8.8μ in diameter. Zoospores biflagellate, with one long posterior and one short anterior flagellum; 4–11, usually 8 or 9, in a sporangium; discharged through papillae formed only on some sporangia on the periphery of the sorus. Resting spores 3.1–4.1μ in diameter, smooth, thick-walled, closely pressed together in circular or ovoid discs one spore thick. Discs usually paired, forming curved, two-layered plates; occasionally in threes or fours, giving an appearance in section of Y- or H-shaped figures respectively.

Sporangial and resting spore membranes without cellulose, as shown by a negative reaction with chlor-iodide of zinc.

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COLLECTION DATA

This organism appeared early in 1949 in five separate collections from Florida and South Carolina. Twenty-nine samples of soil were sent from Florida in February, by Dr. Coker, and were investigated for watermolds by placing them in petri dishes, adding charcoal-treated water, and baiting with halved hemp seeds. Two out of the twenty samples investigated by the writer, and one out of the nine investigated by Mr. W. R. Pendergrass, were found to contain this species of *Sorodiscus*. Mr. Pendergrass also found it in two samples of soil that he collected in South Carolina. Details of the collections are given below.

Collected by Dr. Coker at Fort Lauderdale, Florida:

1. Florida #16. Grassy lot under paw-paw, Second Street, S. W.
2. Florida #17. Cow and horse pasture, under cypress.
3. Florida #27. Near the edge of Flamingo Pool, Rare Bird Farm.

Collected by Mr. Pendergrass in the City Municipal Park, Florence, South Carolina:

4. S. C. #324. Edge of ditch containing slowly-running water.
5. S. C. #333. At a small distance from the above ditch.

METHODS OF CULTURE

Sample #16 of the Florida collection was selected for study. The growth on the hemp seed was removed to another dish and washed well in a stream of sterile, charcoal-treated water. A stock supply of cultures was maintained by renewing the water at intervals, and adding newly-boiled halved hemp seeds, with an occasional transfer to fresh dishes as it became necessary. This was ordinarily carried out at the temperature of the laboratory. During the summer, however, the cultures were dispersed into cooler places, namely, a constant temperature chamber maintained at 18° C., a cold room at 10° C., and a laboratory in the basement of the building. The original soil samples have also been kept, in a dry state. When portions of samples #16 and #17 were rebaited early in 1950, the *Pythium* appeared again with its parasite. The parasitized *Pythium* has not been isolated from the South Carolina samples, but has been kept alive in both by periodical additions of fresh water and hemp seeds.

At first, in order to obtain cultures as free as possible from other micro-organisms, small portions were cut from a vigorous infected growth, washed well, and transferred to clean water containing freshly-boiled hemp seeds. While the *Pythium* was establishing itself on the new seeds, and growing out into a mycelium, a considerable lapse of time was entailed, during which such micro-organisms as were present might multiply extensively, especially at warmer temperatures. The following method, by which clean mycelium is presented for direct infection by the parasite, was therefore tried, and found most successful: The host, previously isolated on agar, is inoculated on 3 per cent. plain agar, and a very small block containing the clean mycelium is placed in a drop of sterile water in a petri dish, with a sterile half hemp seed on top of it. The use of only a drop of water at this stage serves to hold the seed in position on the agar. After about two days, the culture is flooded with enough water to cover the bottom of the

dish. The inoculum is chosen from a culture containing numerous sporangia of the parasite, a portion of which is cut off, and dragged over or through plain agar by means of a sterile needle, for a time long enough to free it from most of the contaminating organisms without seriously damaging it. When it is placed in the dish with the culture of *Pythium*, the spores released from it infect the *Pythium* hyphae, and within eight days, often after four or five, the culture is found to contain large numbers of zoosporangial sori of the parasite in an active state. This stage is reached sooner if sterile soil-water is used instead of distilled water, but contamination also is promoted.

Attempts to transfer the parasite by growth on agar, within the hyphae of the host, have met with no success.

THE IDENTIFICATION OF THE HOST

The host has been isolated from sample #16, and kept in pure single-spore culture. It grows rapidly and produces abundant large spherical sporangia, each with a central vacuole and a small distal "beak". The sporangia are renewed by internal proliferation. For the purpose of measurement, the cultures were grown on a variety of substrates. These included hemp seeds, corn grains, paspalum grass leaves, and flies, in charcoal-treated distilled water, soil-water, and water in which oak leaves had been boiled. Furthermore, measurements were made of sporangia taken directly from a culture in a rebaited portion of soil sample #16. The larger average sizes were promoted in soil-water and oakleaf-water. The measurements were plotted, and the average range was determined graphically. Of five hundred measurements, the total range was 22.5–42.2 μ. The great majority of the sporangia, however, had a diameter of 28.9–32.8 μ.

In order to measure the zoospores, a portion of an active culture in charcoal-treated water was transferred to a drop of fresh water on a slide, and the encystment of the zoospores that were soon released was promoted by drawing off some of the water, and placing a coverslip on the drop. In this way a large number of spores were obtained at the same stage, for measurement immediately after encystment, before the beginning of the swelling associated with germination. A hundred spores ranged from 8.9 μ to 10.6 μ in size, most of them having a diameter between 9.6 μ and 10.3 μ.

For a long time, no oogonia were observed either in the original single-spore isolate, or in the mass-isolate from a re-baiting of soil sample #16. Cultures were grown in distilled water, tapwater, and soil-water, on hemp seeds, corn grains, grass leaves, flies, etc., and on cornmeal, oatmeal, maltose-peptone, and plain agars, without the formation of any oogonia. Neither the addition of small amounts of soil, as recommended by Middleton (1943, p. 9), nor the use of carrot agar, as recommended by Johann (1928), had any effect in promoting their formation. Finally, however, a small number of oogonia appeared in a water culture of the single-spore isolate, around a corngrain embryo that had almost dried. All attempts to obtain more oogonia in the same way have failed. The oospores measured 18.75–26.25 μ in diameter, mostly about 22 μ.

It would appear from the variety of measurements given by different authors

that a number of strains, at least, have been included under the name *P. proliferum* de Bary. The present fungus seems to be one such strain, and has been identified as this species. In the four other collections, the hosts have not been isolated, but it seems that they also are *P. proliferum*.

HOST RELATIONSHIPS

The infected *Pythium* grows rapidly on hemp seeds in water, and produces abundant vigorous sporangia which discharge in the normal way. After about 4 or 5 days, however, the presence of the parasite becomes increasingly apparent. The growth of the mycelium is inhibited, so that the total radial extent of the culture is much reduced compared with that of an uninfected one, and more and more of the swellings can be seen to be occupied by a plasmodium instead of the protoplasm of the host, frequently in an excentric position against the wall. Eventually, the great majority of the swellings contain the zoosporangial sori of the parasite, and soon after, when most of the zoosporangia have discharged their spores, the cystosori appear. By this time, the mycelium of the host has stopped growing; yet, even at this stage, a number of *Pythium* sporangia are always present and may be readily aroused from their dormant state by washing with clean sterile water, and thus provoked into the discharge of active zoospores. In addition, washing in this way usually induces renewed growth of the mycelium by branches especially from the regions of the hyphae near or in the substratum, which were also, presumably, in a dormant condition. It seems, therefore, that the host-parasite relationship is an admirably well-balanced one, since the parasite does not attain ascendancy over its host to the extent of destroying it, nor does the host, once infected, ever grow free of its parasite, in water cultures. On the contrary, cultures containing the host and parasite may be grown and maintained with a minimum of attention, without risking the loss of either one or the other, and thus provide exceptionally favorable material for study.

In attempting to inoculate other species of *Pythium*, this vigor of the original host proves in fact to be a handicap. Thus, if a small portion of infected mycelium is placed in a dish with another *Pythium* species, on a hemp seed, the *Pythium proliferum* establishes itself also on the same substratum and so outgrows the other that it is impossible to determine whether infection has occurred or not. This necessitates the following method: About half a dozen clean cultures of the species of *Pythium* under investigation are placed separately in petri dishes containing sterile water. An infected culture containing large numbers of zoosporangial sori is chosen, washed in a stream of sterile water, and a portion of it is cut off and transferred to a plate of 3 per cent. plain agar. Using a high-powered binocular dissecting microscope, a zoosporangial sorus is dissected out with sharpened needles and rolled over and over on the agar in order to free it from any *Pythium* zoospores that may be adhering to it. A tiny portion of agar, bearing the sorus, is then cut out and transferred to one of the dishes containing the *Pythium* cultures. This is repeated, until all the dishes are inoculated except one, which is kept as a control. After about four days, the cultures are examined at

intervals in order to determine whether infection has taken place. Many times, to see whether the sori treated in this way were still capable of discharging their spores, the above method was adapted as follows: The *Pythium* culture was placed in a large drop of water on a sterile slide in a petri dish, and the agar with the sorus was transferred to the drop. Next day, the sorus could easily be examined under the microscope, and was usually seen to consist of empty zoosporangial cysts. The culture was then completely flooded and left for three or four days more before examination for signs of infection. A number of species with spheroid, lobulate, or filamentous sporangia have been treated in this way, but infection has been observed in only three, *P. undulatum*, *P. irregulare*, and *P. elongatum*, all of which form sporangia of the spheroid type. In these species the host-parasite relationship is an extremely unbalanced one; in favor of the parasite, and the infected *Pythium* very soon dies out.

Attempts to inoculate other watermolds are more easily made. Thus, if a portion of an infected culture is placed in a dish containing *Saprolegnia*, for example, the *Pythium* mycelium that develops is readily distinguishable from the hyphae of the *Saprolegnia*, which can be examined for signs of infection without any further refinement of the technique. This method was used with the following: Four species of *Saprolegnia*, six species of *Achlya*, two of *Isoachlya*, two of *Thraustotheca*, *Leptolegnia subterranea*, *Aphanomyces laevis*, *Dictyuchus monosporus* and several sterile strains, *Brevilegnia diclina*, *Brevilegnia linearis*, and *Geolegnia inflata;* also *Apodachlya brachynema* and *Allomyces arbusculus*. None of these watermolds was attacked, although the parasite multiplied abundantly in the mycelium of the *Pythium* placed with them.

Sorodiscus Callitrichis occurs on *Callitriche*, and *S. Karlingii* on *Chara*. Attempts at infection were therefore carried out with *Callitriche heterophylla*, and a species of *Chara*. *Nitella hyalina* and a species of *Vaucheria* were also tried. All these plants were treated in much the same way as the watermolds listed above, by transferring infected cultures to dishes containing a number of filaments or fronds of the green plant. The *Nitella* and *Callitriche* were given special treatment in addition: The *Nitella* was planted in sand in several large beakers of water, in some of which infected cultures were also placed. The *Callitriche* was planted in soil in beakers and fingerbowls of water, and similarly inoculated. The inoculation was repeated several times. At intervals over a period of several weeks, the plants were examined macroscopically for galls or abnormal swellings of any kind, and microscopically for signs of the presence of the parasite within the cells. No such symptoms of infection were observed.

THE DEVELOPMENT OF THE PARASITE

(1) *The zoosporangia*

The mature zoosporangia of *Pythium proliferum* are spherical, papillate, each with a large vacuole in the center. In an infected culture, a plasmodium of the parasite at an early stage of its development may frequently be found in a sporangium. It is distinguished by rather darker, more heavily granular protoplasm, an irregular outline, and numerous, fairly conspicuous threads connecting it

across the widening space with the protoplasm of the host, which is gradually reduced to a thinner and thinner layer as it becomes transported along the threads towards the plasmodium. At first, the limits of the plasmodium may be impossible to define, partly concealed by the mass of granules and globules of the disintegrating protoplasm. However, at maturity it is seen to be surrounded by a delicate membrane. Finally, all that remains visible of the host's protoplasm is a few drops or granules close against the plasmodium, or around the walls of the sporangium. Unlike *Octomyxa Brevilegniae* (Pendergrass, 1948), in which the plasmodium may be lateral at first but later moves to the center of the gall, in this species of *Sorodiscus* it seems that it often remains against the wall, and completes its development in this position. In those instances in which the plasmodium has been seen in a central position, it has also been of a particularly large size relative to the size of the *Pythium* sporangium. Probably, then, the plasmodium takes up a central position only when the sporangium has been infected before having vacuolated, when the entire volume is occupied by protoplasm. The relatively large size of the plasmodium would thus be accounted for.

Indistinct light and dark areas become visible, and the plasmodium becomes cleaved up into zoosporangia. These are extremely variable in size, ranging from 5.5 μ to 8.8 μ in diameter. There is apparently no correlation between the size of the sporangia of the parasite and that of the host sporangium containing them; any one sorus may contain sporangia of opposite extremes in size. There is some correlation, however, between the number of sporangia in the sorus, and the size of the *Pythium* sporangium. Obviously, this number is determined by the size of the plasmodium, which is itself determined by the quantity of the host's protoplasm available for its enlargement; this is limited by the septum at the base of the sporangium. In members of the Saprolegniaceae parasitized by *Octomyxa* no such septum-formation occurs. This difference, together with the fact that mature sporangia of *P. proliferum* usually contain a large central vacuole, may possibly explain why the zoosporangial sorus fills the swelling containing it, more often in *Octomyxa* than in *Sorodiscus*. In a vigorous culture of *Pythium proliferum* in soil-water, in the majority of the sori thirty to forty sporangia may be counted at single level of focus, probably representing only about half the total number of sporangia present. In one of the large ellipsoid sporangia of *P. undulatum*, over sixty sporangia of the parasite have been counted at one level of focus.

Indeed, the successful inoculation of species other than *P. proliferum* has given final, unquestionable evidence as to the identity of the structures containing the sori, which seemed at first to be a problem. Thus, in *Octomyxa Achlyae* (Couch, Leitner, and Whiffen, 1939) and *Octomyxa Brevilegniae*, the plasmodia are found in large spherical swellings totally distinct from any organs normally formed by the *Achlya* and *Brevilegnia* hosts, respectively. These swellings are produced by a hypertrophy of the host caused by the parasite, resulting in the formation of galls. In *Sorodiscus Cokeri* on *Pythium proliferum*, however, repeated measurements have revealed a very close correspondence between the ranges of size shown by the sporangia of healthy, uninfected cultures and by the

structures containing the zoosporangial sori of the parasite. In other words, the latter have not been found to exceed the former in size. It appears, then, that galls comparable with those brought about by *Octomyxa* are not caused by this species of *Sorodiscus*. Rather, the plasmodia of this parasite develop in organs of its host, distinctly recognizable as such. This is especially evident in infected cultures of *P. undulatum*, in which the sori are found in structures of the same large size and ellipsoid shape as those of normal sporangia, but it is also borne out by *P. irregulare* and *P. elongatum*, in which the parasitized structures show a variation in size and shape similar to that of healthy sporangia. Moreover, the parasite has been observed in incipient chlamydospores of *P. undulatum*, and oogonia of *P. irregulare*. Failure to infect species of *Pythium* with filamentous sporangia may perhaps be correlated with the apparent incapability of the parasite to induce the formation of swellings in the hyphae.

Hypertrophy in the usual sense, then, does not occur here. There is, on the other hand, in some infected cultures, a larger proportion of smaller swellings than in healthy cultures. Occasionally, these are intercalary; more usually they are at the tips of the hyphae, and are often elongated, swollen distally, representing either incipient sporangia halted in their development, or precocious swellings, brought about by the parasite. If the second interpretation is accepted, and considering the fact that in normal cultures of *P. proliferum* intercalary swellings are somewhat rare, then hypertrophy may possibly be said to occur, but to so small an extent as to be practically negligible.

(2) *The zoospores*

Newly-formed sporangia, though clearly delimited by a membrane, appear pale, with minute droplets scattered through the protoplasm. As zoospore formation begins, the protoplasm takes on a more refringent, and generally coarser appearance, due to the presence of larger droplets. At the same time, the wall of the *Pythium* sporangium, previously spherical, becomes irregular, slightly lobed, owing to the development of out-growths from the *Sorodiscus* sporangia which press against the wall. This irregularity of outline is more pronounced in host sporangia in which the sorus does not occupy the entire volume than in those that are completely filled by the sorus and are thus subjected to more nearly equal pressures. These outgrowths are the exit papillae of the parasite. They are formed only by some of the sporangia on the periphery of the sorus. They are at first filled with granular protoplasm similar to that of the main body of the sporangium, which has by this time a slightly mottled appearance due to the presence of the relatively large oil drops. The region where the papilla is pressing against the wall of the *Pythium* sporangium is highly refractive. The protoplasm withdraws from the papilla leaving it perfectly hyaline, and cleavage areas become visible in the sporangia, dividing the contents into polygonal portions with bluntly rounded corners. These spore origins become more distinct and individualized as zoospores. Soon, one of them oozes up into the papilla and after a short time emerges, followed in turn by the others, which can be seen previously moving around in the sporangium. The hyaline, amoeboid proto-

plasm of the zoospore flows out first, forming a spherical drop; then the granular protoplasm with its droplets flows into it. Finally the anterior end is drawn out, sometimes with a jerk.

There is some variation in the appearance of the papillae that serve in the discharge of the sporangia. At an early stage, the papilla is somewhat ellipsoid, pressed and flattened against the wall of the *Pythium* sporangium. Soon, a protuberance appears on it, pushing out the confining wall like a minute, delicate bubble. It is the size of this bubble that varies. Thus, while it may sometimes be only a slight projection, it often comes to equal in size the part remaining within the sporangium. In any one sorus, the papillae are of approximately the same type. This variation is probably determined by the solubility and extensibility of the *Pythium* wall, which may differ from one sporangium to another, according to relative age and the state of vigor of the mycelium at the time of their formation, and possibly to other factors, also. Delicate as this bubble appears, it is nevertheless persistent, and after discharge is over, it may sometimes be seen surrounded at the base by a thicker wall, like a short flared collar. It seems reasonable to suppose that this collar represents the *Pythium* wall, at first softened and extended, but finally ruptured, by the pressure of the papilla of the parasite. With the consequent release of pressure, both parts of the papilla, within and without the *Pythium* sporangium, attain an almost spherical shape, and the escape of the spores is initiated.

There are usually seven or eight spores in a sporangium, but a range of three to eleven has been found. Complete evacuation of the entire sorus takes a considerable time. The sporangia do not all mature simultaneously, and when the contents of most are clearly divided up, and some have already discharged their spores, others may be seen still undivided. The spores in the sporangia in the interior of the mass escape by passing from one sporangium to another, squeezing through narrow pores that develop in contiguous walls. Slightly more refractive regions of the walls visible before the escape of the spores may possibly mark these areas.

While some spores swim away almost immediately after emergence, most undergo a period of amoeboid movement in the vicinity of the papilla. They are frequently top-shaped at first, the anterior end being pointed, the posterior end being lobed. Sometimes they are drawn out posteriorly into one or two long finger-like processes. A number of droplets or granules are usually contained in the anterior end. The posterior region is constantly undergoing changes in shape, giving the impression of a flowing or oozing movement. During this movement, the long flagellum may wave slowly back and forth with wide undulations, apparently ineffectively, continuously in view throughout its length. The short flagellum is more active, and may undergo vigorous beating movements which seem to be responsible for the rapid oscillations that develop. After a time, the spore assumes a regular, streamlined shape and swims away, a little jerkily at first, but finally smoothly and rapidly, with the long flagellum directed posteriorly.

(3) *The cystosori*

The cystosori appear later in the developmental cycle, when most of the zoosporangia have discharged. At room temperatures, they are formed more abundantly in the summer than the winter. The plasmodia forming them are not at first very easily distinguished from those forming zoosporangia, except that, as in *Octomyxa*, they have somewhat denser, darker protoplasm. Also, the protoplasm does not become heaped against the wall, or even in the center, but usually forms a continuous curved plate across the sporangium. The protoplasm develops a spongy appearance, indistinctly vacuolate; then the outlines of the spores become visible and more and more distinct as the spore-membranes thicken. The absence of a plasmodial membrane throughout the entire process marks an essential difference in the development of the cystosorus from that of the zoosporangial sorus. Usually, the final configuration is that of two discs closely pressed together, forming a "spore-cake" (Lagerheim and Winge, 1913, p. 17), with no space between, other than that due to small differences in the sizes of the spores. This double disc often extends across the middle of the lumen of the sporangium, with only a slight curvature, if any, and appears in side view as a dark band of spores in two rows. In face view, the sorus appears as a circular or elliptical plate of spores pressed tightly together throughout, except for a variable number of irregular spaces. In both views, the spores appear angular, as a result of pressure, but their free surfaces are rounded. Their walls are thick and slightly brown; their contents are granular. Their measurements vary from 3.16 by 3.95 μ to 3.63 by 4.10 μ. The number of spores in a disc is variable, but it is usually around sixty, in cultures in soil solution made from the original sample of soil. In this solution, the diameter of the sori has been found to range from 15.12 μ to 33.6 μ, most of them measuring 25.2 μ to 30.2 μ. Obviously, these sizes are determined largely by the dimensions of the *Pythium* sporangia containing the sori.

A sorus consists quite often of three discs, and sometimes of four. In the three species successfully infected, which form sporangia of widely different shapes and sizes, the discs are found in various arrangements, and are sometimes separate from one another. On the other hand, in *P. proliferum*, which forms spherical sporangia, the discs are almost always closely associated, as though derived from a single plasmodium. No evidence that more than one plasmodium may take part in the formation of a sorus has been observed. However, one instance has been seen in which a single *Pythium* sporangium contained both a cystosorus and a sorus of zoosporangia, a condition comparable with that observed once in *Octomyxa Achlyae* (Couch, Leitner, and Whiffen, 1939, p. 402).

At no time has a soral membrane been seen. Cystosori have been tested with a number of reagents, including iodine in potassium iodide, iodine and sulphuric acid, and caustic potash, without revealing any indication of the presence of a common membrane. Considering the absence of a plasmodial membrane throughout the development of the cystosorus, the possible seat of origin of such a common soral membrane is hard to conjecture.

In old cultures, inevitably swarming with bacteria and protozoa, many of the

cystosori are freed by the disintegration of the *Pythium* walls containing them. Even under these conditions, however, they usually remain intact, the two discs of the spore-cake still adhering together. The liberation of sori consisting of three or four discs does usually result in the release of separate single-layered plates.

As in *Octomyxa Brevilegniae*, attempts to control the germination of the resting spores have so far failed. Wholly empty sori, and sori containing a few empty cysts, have been seen. Also, infection of fresh *Pythium* mycelium can be secured in cultures that have been kept unchanged for several months, and are thus unlikely to contain zoosporangia. The germination of the spores, however, has not been observed.

CYTOLOGY

The nuclear history has not yet been investigated in detail, and in view of the existing confusion in the literature on the behaviour of the nuclei in the Plasmodiophoraceae, no opinions will be expressed here. Suffice it to say that cruciform divisions in plasmodia undergoing vegetative growth and mitotic divisions at the zoosporangial stage have been demonstrated. For these investigations, growths on hemp seeds were fixed in Randolph's Modified Navashin Fluid (Johansen, 1940, p. 45) and stained *in toto* according to a schedule[1] based on a modification of Gram's gentian violet technique communicated to me personally by Miss Leitner. Small portions of the stained material were teased off and carried through the final stages of the procedure.

Zoospores were fixed by exposure to osmic acid fumes and stained with gentian violet, according to the method described by Cotner (1930), which serves excellently to show up the short anterior and long posterior flagella.

DISCUSSION

The new species described above has many points of resemblance to the two species of *Octomyxa*, the only well-established members of the Plasmodiophoraceae which have been found as parasites of fungi. The stages in its development agree closely with those of *Octomyxa Brevilegniae* as described in detail by Pendergrass (1948, 1950). That it is, on the other hand, very distinct from *Octomyxa* is shown by the following outstanding differences in their morphology, physiology, and effects on their hosts: Firstly, the cystosorus is characteristically in the form of a spore-cake consisting of two closely adpressed discs, while in *Octomyxa* it is a spherical mass of spores associated in groups of eight. Secondly, the host range, so far as known, is limited to certain members of the genus *Pythium*, and Saprolegniaceae are not attacked, while both species of *Octomyxa* parasitize some members of the Saprolegniaceae only. Finally, the parasite de-

[1] Staining schedule: Material fixed in Randolph's Modified Navashin Fluid and stored in 70 per cent. alcohol was brought down to water and treated as follows: *a*. 0.5 per cent. aqueous solution of gentian violet, 24 hours. *b*. Water, 5 minutes. *c*. Iodine solution, 3 minutes (iodine, 1 gm.; potassium iodide, 2 gms.; water, 300 c.c.) *d*. Water, 5 seconds to an indefinite time. *e*. 95 per cent. alcohol, 5 seconds. *f*. Absolute alcohol, 30 seconds. *g*. Clove oil, about 20 seconds. *h*. Cedar oil, 1 minute. *i*. Canada balsam.

velops in structures delimited by septa, closely resembling normal organs of the host, without causing obvious hypertrophy, while the formation of true galls, not cut off by septa, is caused by both species of *Octomyxa*. It is clear, then, that the new parasite cannot be classed with the other two species under the name *Octomyxa*.

Another new parasite of *Pythium*, to be described in a later paper, belongs to the genus *Woronina*, which is placed in the Plasmodiophoraceae by Sparrow (1943), but has not so far been shown conclusively to belong to this family. From this organism also, the species described in this paper is entirely distinct. The latter, therefore, cannot be placed with any of the other parasites of fungi so far described.

The genera of the Plasmodiophoraceae are distinguished according to the form of the cystosori. On this feature, the new organism falls into the genus *Sorodiscus*, as defined by Winge (1913), in which the spores "arrange themselves in spore-cakes two layers thick, or one might say—perfectly flattened hollow spheres or hollow ellipsoids." The genus contains three established species: *S. Callitrichis* Lagerheim and Winge, on two species of *Callitriche; S. radicicolus* Cook, on *Gynandropsis pentaphylla;* and *S. Karlingii* Cook, on two species of *Chara*. It is suggested by Karling (1942, p. 52) that *Sorodiscus Heterantherae* Wernham is identical with *Membranosorus Heterantherae* Ostenfeld and Petersen. A zoosporangial stage has been seen in none of these species. Its occurrence will possibly be revealed by further observations of living material. These are necessary, in any case, to complete the rather inadequate descriptions, and clarify many of the questionable points raised in them and discussed by Karling (1942, p. 4), such as the presence or absence of a common soral membrane. In *Sorodiscus Cokeri* no such membrane is present.

This species differs consistently from the other members of the genus not only in being a parasite of fungi, but also in causing no obvious hypertrophy of the host; furthermore, in a single portion of the host delimited by septa it is unusual to find more than one sorus, while in the other species a single cell of the host frequently contains several sori, in the case of *S. Karlingii* up to four hundred. The last two differences are probably closely related to the first, and determined by the entirely different organization of the two types of host. For the hosts of *S. Cokeri* are coenocytic, while those of the other species are divided by cellulose walls into uninucleate cells interconnected, presumably, by plasmodesmata. Thus, while the *Pythium* hosts form, in their normal course of growth, swellings perfectly suited to the development of a single plasmodium, to which an unlimited supply of protoplasm might at first appear to be available from the flow of protoplasm along the hyphae, the formation of impermeable septa, also a normal function of the host, does in fact completely and finally limit this supply.

This wide variation in hosts is indeed remarkable, but not without parallel. Thus, in a family of chytrids, the Synchytriaceae, the species of *Synchytrium* attack only flowering plants, while those of *Micromyces* and *Micromycopsis* occur on algae. The members of the three genera are morphologically similar, funda-

mentally, and undergo the same characteristic developmental stages, yet their occurrence on such dissimilar groups of plants, indicating great differences in their physiology, is considered of sufficient importance to justify their separation into different genera. However, despite the physiological tendencies outlined in a recent paper by Cantino (1950), it is the opinion of the present writer that there is increasing evidence that physiological differences among the lower organisms are specific rather than generic.

There seems, therefore, to be no reason why the organism described in this paper should not be included in the genus *Sorodiscus*. The configuration of the cystosorus would seem to be a far more reliable generic feature than the nature of the host. Indeed, the highly characteristic occurrence of the resting spores of this species in single-layered discs usually associated in pairs, forming spore-cakes which cannot very easily be conceived of as derived from flattened spheres or ellipsoids, provides concrete evidence of the validity of the genus. This is contrary to the theory of Palm and Burk (1933), based on the variation in *Sorosphaera Veronicae*, that the form of the cystosorus is not a reliable taxonomic character, and their proposal that six genera of the Plasmodiophoraceae, including *Sorodiscus*, should be considered synonymous and united into one large genus. On the other hand, agreement is found with Winge, who, believing at first (1913) that *Sorodiscus* showed such similarity to *Sorosphaera* "that it would seem most reasonable to unite them into one genus", later communicated to Ivimey Cook (1931) that "the spore masses are so characteristic in *Sorodiscus* that it would be wrong to put it in the genus *Sorosphaera*."

The identification of the organism described in this paper as a new species of *Sorodiscus* to be called *S. Cokeri*, seems, then, to be amply justified.

SUMMARY

Sorodiscus Cokeri on *Pythium proliferum* de Bary is described as a new species, the first member of the genus in which zoosporangia have been seen, and the only one so far known as a parasite of fungi. The plasmodia develop chiefly in the sporangia of the host, causing little or no hypertrophy. Cruciform nuclear divisions take place during vegetative growth. At maturity, a sorus of zoosporangia or resting spores is formed. The zoosporangia are thin-walled, spherical or ellipsoid, forming heterokont zoospores, each with a long posterior and a short anterior flagellum. Exit papillae are formed only by some of the sporangia on the periphery of the sorus. The zoospores formed in the interior of the sorus escape by passing through narrow pores formed in contiguous walls, from one sporangium to another, and thus to the outside. In its developmental stages this organism closely resembles *Octomyxa*. It is characterized, however, by its cystosori, which are composed of one-layered discs of resting spores. Usually there are two discs in a cystosorus, closely associated to form a spore-cake. Frequently there are three or four, forming Y- and H-shaped figures, respectively, in sectional view. This species is limited in its host range to *Pythium*, of which three other species have been successfully infected. These are *P. irregulare* Buisman, *P. undulatum* Petersen, and *P. elongatum* Matthews.

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PLATE 1

Sorodiscus Cokeri on *Pythium proliferum*

FIG. 1. Binucleate plasmodium in hyphal tip, with nuclei undergoing cruciform division. × 1700.

FIGS. 2–3. Nuclei undergoing cruciform division. × 2800.

FIGS. 4–7. Plasmodia developing in terminal and intercalary swellings of host hyphae; plasmodia appear darker than host protoplasm. Fig. 4, plasmodium in lateral position. Fig 5., plasmodium drawing away from wall into a central position, connected with peripheral host protoplasm by delicate strands. Fig. 6, lateral plasmodium barely distinguishable on outer side from host protoplasm. Fig. 7, young plasmodium in elongated intercalary swelling. × 1055.

FIG. 8. Plasmodium showing the vacuolation that precedes cleavage into zoosporangia. × 1055.

FIG. 9. Similar stage to fig. 8. The two blunt projections represent aborted outgrowths of the Pythium sporangium. × 730.

FIG. 10. Plasmodium undergoing cleavage into zoosporangia. × 1055.

FIG. 11. Sorus of newly-formed zoosporangia; note unusually large size. × 1055.

FIG. 12. Early stage in formation of zoospores. Protoplasm withdrawn from papillae, leaving them hyaline. × 1055.

FIG. 13. Cleavage of sporangial contents into zoospores. × 1055.

FIGS. 14–16. Successive stages in the same sorus: Fig. 14, 11.10 a.m., sporangia are fully formed; one has developed a papilla. Fig. 15, 11.30 a.m., host wall has an uneven contour due to the pressure of the papillae; regions of host wall in contact with papillae are highly refractive. Fig. 16, 3 p.m., cleavage into zoospores is taking place; papillae are hyaline owing to withdrawal of protoplasm. × 1055.

FIG. 17. The emptying of the zoosporangial sorus, with zoospores passing from one sporangium to another, escaping through openings formed by papillae, and undergoing amoeboid changes of shape before swimming away. A contractile vacuole, a number of scattered droplets, and a long refractive body may be seen in the zoospore. × 1340.

FIGS. 18–20. Empty zoosporangial sori. × 1055.

PLATE 2

Sorodiscus Cokeri on species of *Pythium*

FIGS. 21–24. Cystosori in sporangia of *P. irregulare*: Fig. 21, sorus composed of 2 discs. Fig. 22, sorus composed of 3 discs. Figs. 23–24, sori composed of 4 discs. × 592.

FIG. 25. Cystosorus composed of 3 discs in oogonium of *P. irregulare*. × 592.

FIGS. 26–29. Cystosori in sporangia of *P. undulatum:* Fig. 26, cystosorus composed of 4 discs; an empty zoosporangial sorus is contained in a small sporangium produced by branching below the larger one. Figs. 27–28, sori composed of 3 discs; in fig. 28 an additional septum has been formed. Fig. 29, sorus composed of 4 discs. × 592.

FIG. 30. Cystosorus composed of 2 discs in chalmydospore of *P. undulatum*. × 592.

FIGS. 31–39 all refer to *P. proliferum:*

FIG. 31. Plasmodium in diametrical position, connected with peripheral host protoplasm by delicate strands; vacuolation is apparent. × 1055.

FIG. 32. Plasmodium occupying about half a sporangium, indistinguishable on one side from the host protoplasm; faint indications of cleavage already visible. × 1055.

FIG. 33. Newly-formed resting spores lying across a sporangium in 2 closely adpressed discs, only the edges of which are figured. × 1055.

FIG. 34. Mature cystosorus composed of 2 flat discs. × 1055.

FIG. 35. Cystosorus composed of 2 deeply convex discs, separated on the far side of the sporangium. × 1055.

FIG. 36. Cystosorus composed of 3 discs. × 1055.

FIG. 37. Similar cystosorus to fig. 35, in marginal view. × 1055.

FIG. 38. Face view of disc freed by disintegration of the host wall, showing a few irregular spaces among the otherwise compact resting spores. × 1055.

FIG. 39. Part of empty cystosorus, showing exit openings of some of the spores. × 1700.

PLATE 1

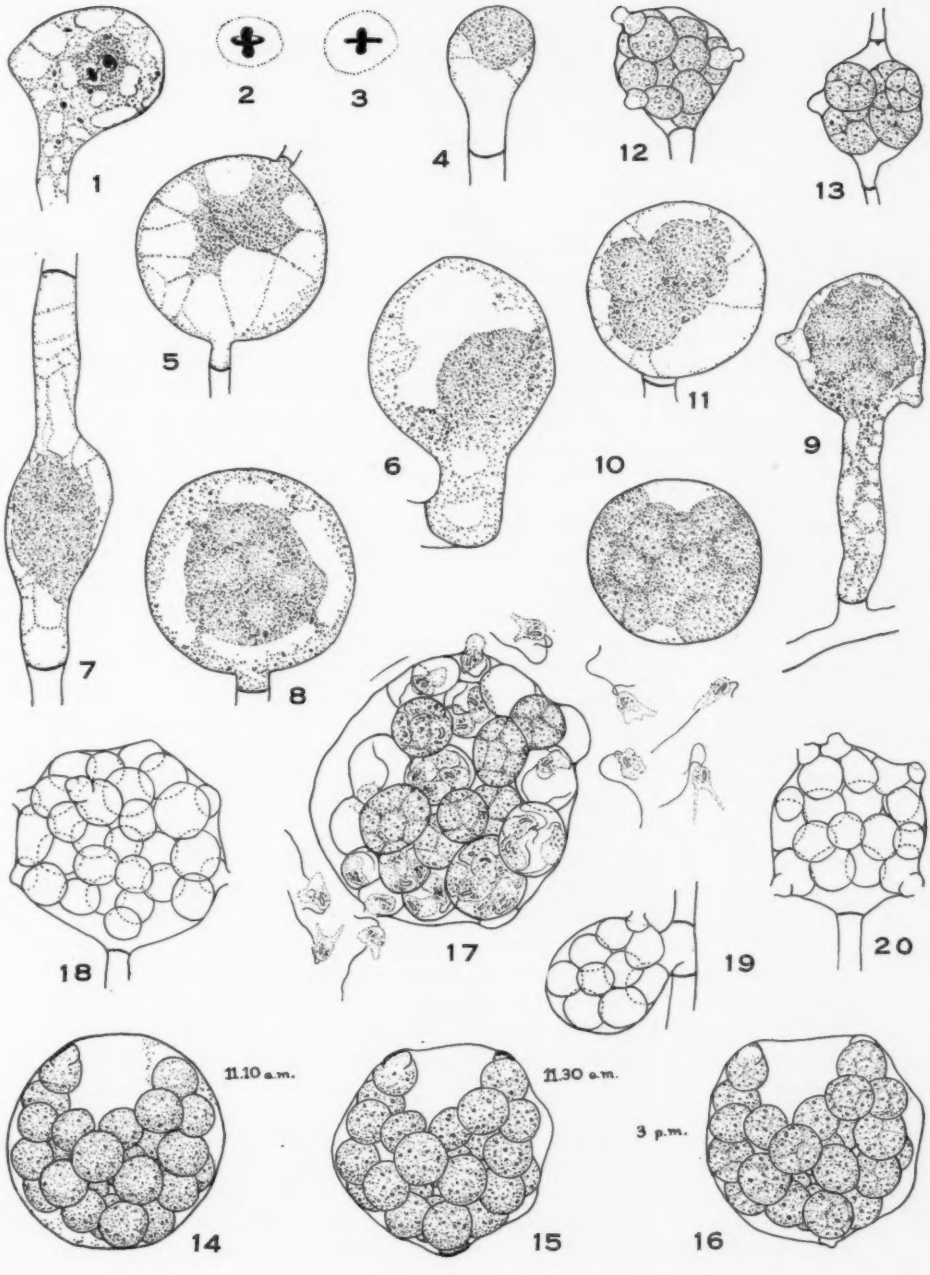
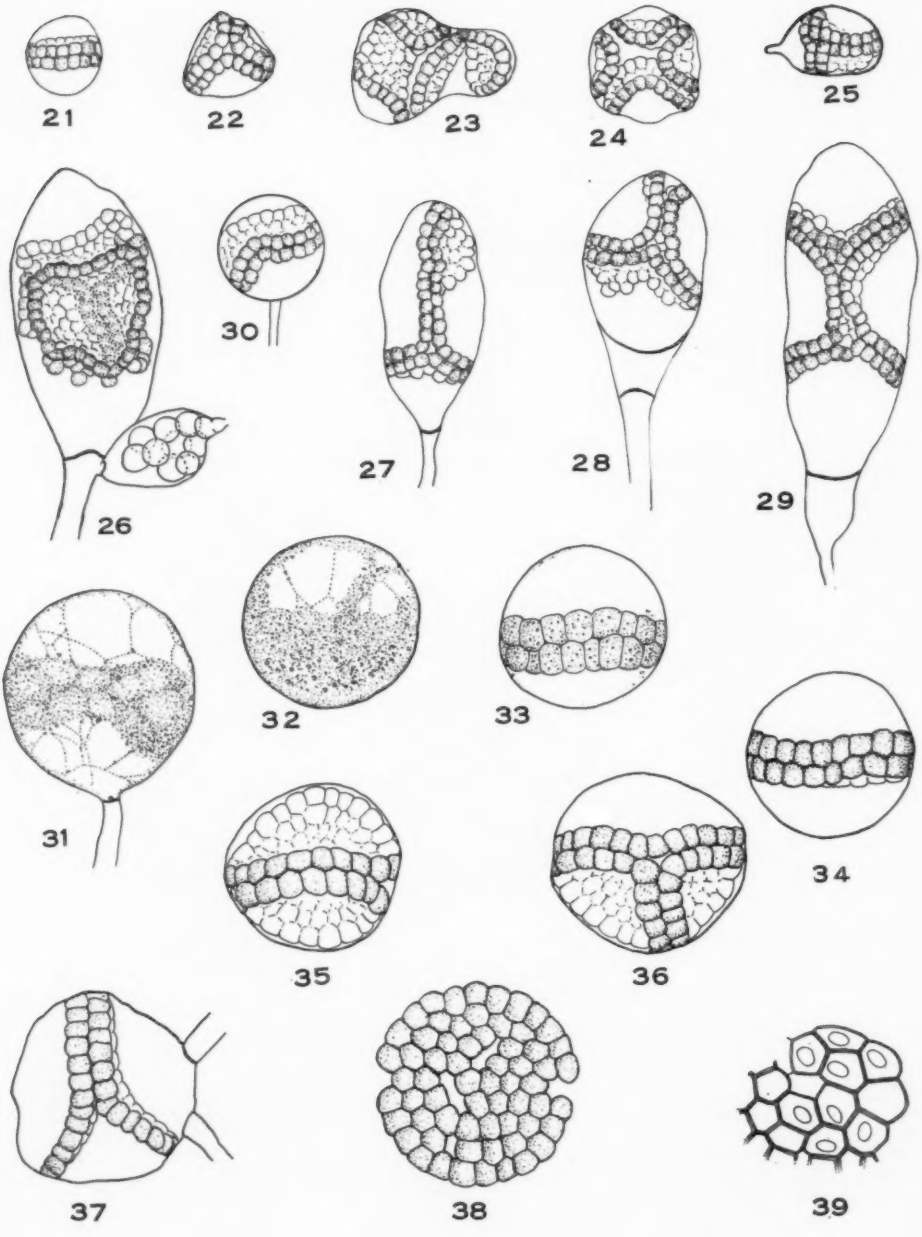


PLATE 2



A STUDY OF THE MOTILE CELLS OF VAUCHERIA

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Plates 3–5

The systematic position of *Vaucheria* has long been in question. Although the genus was removed from the green algae (Chlorophyta) by Bohlin in 1901 and by Blackman and Tansley in 1902, and placed in the yellow-green algae (Xanthophyceae), this transfer was not accepted subsequently. *Vaucheria* was considered Xanthophycean by these authors on the basis of 1) chloroplast pigments, 2) type of stored food, and 3) form of the spermatozoid. Recently the first of these features was rather conclusively pointed up in the pigment analyses of Strain (1948). In fact, it was mainly because of Strain's work that Smith (1950) transferred anew *Vaucheria* from its traditional place in the Siphonales of the Chlorophyta to the Xanthophyceae.

During the past half century the individual motile cell has become increasingly important in the classification of the algae and lower fungi, with special interest on the number, relative lengths, position of attachment, direction, and structure of the flagella. Since Löffler's (1889) description of tinsel and whip lash types of flagella, flagellar structure has proved to be a useful and significant feature. Vlk (1938) summarized his own studies along with the previous work of others, showing that in the major groups of algae flagellar structure is constant. Couch (1941) confirmed and extended conceptions of the organization of the lower Phycomycetes on the basis of his studies on flagellation. Vlk (1938) and Brown (1945) have given excellent historical accounts of this field of study, and Owen (1947) analyzes the fixation and staining techniques.

Although a number of observations have been made on the motile cells of *Vaucheria*, no special study has been applied to the spermatozoid and zoospore with respect to flagellation, and flagellar structure has never been determined. For this reason, at the suggestion of Dr. J. N. Couch, whose interest in the problem was renewed through a letter from Dr. Gilbert Smith in 1948, a study of the motile cells of *Vaucheria* was begun. It was expected that this study would shed some light on the controversial taxonomic position of the genus, as well as on the questionable relationship that has been frequently supposed between *Vaucheria* and the Phycomycetes as represented by *Saprolegnia*.

Materials and Methods

In the course of collecting and handling *Vaucheria*, several interesting observations incidental to the study were made. All of my observations on terrestrial *Vaucheria aversa* Hassall, *V. pachyderma* Walz, *V. borealis* Hirn, and *V. sessilis* De Candolle show that their antheridia and oogonia hang down or tend to be directed toward the center of the earth (figs. 1, 2). Thus, in the field it is difficult

to tell with a 10X hand lens whether or not sexual organs are present. This is not the case with *Vaucheria geminata* De Candolle, *V. hamata* (Vaucher) D. C., and *V. terrestris* Lyngbye em. Walz, which bear their sexual organs on short branches sticking up, away from the surface.

A most interesting pattern of growth was observed for the vegetative filaments of *Vaucheria*. Observations on terrestrial collections are first made with a dissecting binocular microscope by reflected light. It is then often obvious that the filaments extend in a spiraling way. Sometimes the spirals are tight, giving the filaments a somewhat beaded appearance; at times the spirals are loose and quite obvious; then again spiraling may be so loose or irregular that one can only tell that the filament's growth is not in a directly straight line. With this knowledge, careful observations by transmitted light on aquatic specimens indicate the same. Discharging zoospores when they gyrate out (fig. 9), swimming spermatozoids and zoospores, and the antheridia of some species also show this spiral character. Thus, there is evidence that the spiral pattern of growth is the basic pattern for *Vaucheria*.

Species identification for zoospores was made in the following way: into a clean petri dish sand was put at an incline and partly covered with distilled, filtered charcoal or spring water so that some sand was above the water. A small portion of the *Vaucheria* material which had just produced zoospores was placed on the sand, part of it above and part below the surface of the water. The petri dish was covered and put on an outside window ledge, where it was cool and in open sunlight. Sex organs generally formed in a week, thus permitting identification.

For making observations on spermatozoids and preparatory to staining them, each mount was made in the following way: a single *Vaucheria* filament on which there were maturing sex organs was pinched off by means of needles. With a small pipet this filament was then transferred to distilled, filtered charcoal or filtered spring water. It was washed in this water several times by means of successive pipetting in order to remove contaminants, especially protozoa which might be confused with *Vaucheria* spermatozoids. The filament thus cleaned was mounted in a drop of water on a clean glass slide for observation.

Spermatozoids and zoospores which were to be studied for flagellar structure were killed by exposure to the fumes of 1% osmium tetroxide solution for 15–30 seconds and fixed by drying on the microscope slide at room temperature. Those zoospores which were destined to be sectioned were killed and fixed in weak Flemming's solution for 24–48 hours, washed in water for 24 hours, run through a closely graded ethanol series into chloroform, and then imbedded in paraffin. The zoospores were sectioned at 4–6μ and stained with both crystal violet (modified Gram's stain) and 0.5% Haidenhain's hematoxylin, mordanted and destained with 2% iron alum.

The Löffler technique for bringing out flagellar structure, and the quick method for staining whip lashes with crystal violet, always employed along with the Löffler technique, were essentially the same as Couch's (1941) methods.

SPERMATOZOIDS

The spermatozoids of *Vaucheria* are now generally described as exceedingly small, oblong or pear-shaped, colorless bodies provided with two laterally inserted flagella, one directed forward and the other oppositely behind. It may be that Woronin's (1869) figures, which are the only ones that stand out clearly and convincingly, have had some influence on the failure to give recognition to the concordant observations of Pringsheim (1855), Walz (1866), and Strasburger (1889), who found that in a number of species the two flagella of a spermatozoid are unequal in length. Their observations were made after killing and staining with iodine. The only figures showing this, however, and these are exceedingly small, are those of Pringsheim for *V. sessilis* and Walz for *V. pachyderma*. Walz states that the spermatozoids "possess two unequally long cilia, one of which is directed forward, the other backward, and both of which arise from a point near the front end of the spermatozoid."

Gross (1937) has found for *V. geminata* that the living antherozoid "has a red pigment spot and two cilia of apparently equal length attached toward the anterior end." Whereas, in 1939 P. Dangeard finds that the living antherozoid (of *V. geminata*) is completely colorless, lacking a stigma and provided with two flagella which are very long, essentially equal and laterally inserted.

My darkfield observations of the swimming spermatozoids of *V. pachyderma* and *V. aversa* show only that the two flagella are oppositely directed, that they are both very active, and finally that the actively swimming spermatozoid apparently rotates on its axis as it moves forward spirally. That the flagella are not apically inserted can be seen on those spermatozoids which are not actively moving. Brightfield observations on relatively inactive spermatozoids of *V. pachyderma* show at times a globule of slightly different refractiveness from the cytoplasm, which about half fills the spermatozoid (perhaps this is the nucleus); at times one or two smaller and more highly refractive granules are obvious. It is clearly seen that the pointed end of the spermatozoid is directed forward. The spermatozoids killed with osmium tetroxide fumes average about 5.5μ long and 2.5μ wide at the back or broadest end.

When the spermatozoids of *V. sessilis* are killed with osmium tetroxide fumes, dried on the microscope slide, and then stained for 15–30 seconds with an aqueous solution of crystal violet, which is so weak that the solution is only faintly tinted, their internal structure is apparent (fig. 7). After so treating, about one-half of the spermatozoid is seen to be occupied by the nucleus. Within the cytoplasm there are a few lighter and a few darker-staining globules. The sub-apical or lateral attachment of the flagella is obvious now by the presence of a basal granule (in reality probably two). The lightly stained flagella show no obvious difference in length or structure.

After the spermatozoids of *V. sessilis*, *V. pachyderma*, *V. aversa*, and *V. geminata* are deeply stained with crystal violet or by the Löffler procedure, the two flagella are seen to be of different lengths, the longer one with a whip lash, the shorter one blunt and, in the case of *V. pachyderma*, apparently with tinsels

(figs. 3–8, 18). It is almost certain that it is the longer, whip lash flagellum which is directed posteriorly; for with all flagellated cells which have been so studied, the flagellum which is directed backward is of the whip lash type. It follows that the shorter flagellum is the one which is anteriorly directed. Keeping in mind that it is the pointed end of the spermatozoid which is anterior, one can see that this is true from looking at the included figures.

The most satisfactory technique for showing tinsels is that of Löffler, and even with this results are often erratic, effective staining varying not only from slide to slide but even among cells on the same slide. The shorter, blunt flagellum of *V. pachyderma* is probably a tinsel flagellum (figs. 4, 18). Sometimes the tinsels are seen clearly, then again they are often only apparently there; sometimes there is no evidence of tinsels. This shorter flagellum is at times a little thicker than the whip lash flagellum; for one preparation this was considered characteristic. The tip is relatively blunt, though sometimes somewhat pointed rather than rounded. My Löffler preparation of *V. sessilis* fails to reveal tinsels on the shorter, anterior flagellum. Besides differing from the posterior, whip lash flagellum by having a blunt rather than pointed tip, the shorter flagellum is here usually thicker and often with decidedly more irregular edges (fig. 5). It may be that this greater thickness and roughness is correlated with the tinsel type of flagellum when prepared by this method.

It may be said, in conclusion, that the spermatozoids of *Vaucheria* are distinctly heterokont, their two laterally inserted and oppositely directed flagella differing both in length and in structure. There is evidence that the shorter, anteriorly directed flagellum is of the tinsel type. The longer, posteriorly directed one is a whip lash flagellum.

Zoospore (Synzoospore)

Schmitz in 1879 found that in the outer colorless layer of protoplasm of the zoospore of *V. sessilis* there is a single layer of nuclei, and that "with each nucleus there is a corresponding pair of cilia, which arise from the surface of the colorless plasma layer and originate in pairs from a small, thick nodule on the surface." (Taken from Strasburger, 1889.) Strasburger (1889, 1892, 1900) confirmed Schmitz' observations and made more extensive studies on the zoospore of *V. sessilis*. He stained both *in toto* and after sectioning.

The pairs of flagella are considered as equal in length. There is only the conjecture of Pascher (in a footnote in Heering's taxonomic treatment of the Siphonales in 1921), who doubts that in the case of the synzoospores pairs of equally long flagella are present, and considers that two unequally long flagella are not out of the question. P. Dangeard (1946) states that he has found that the flagella of the zoospore are quite equal in length.

The zoospores of *V. geminata* and *V. pachyderma* are large and when actively swimming are oblong to ovoid (fig. 10). There is a large, conspicuous vacuole in the anterior part (fig. 10) so that the chloroplasts are confined to a more or less single layer just beneath the layer of nuclei (fig. 12). This large vacuole is occasionally nearly central in position; at times there may be two large, con-

spicuous vacuoles. Sectioned zoospores of *V. pachyderma* show that the posterior, denser part contains as well numerous small vacuoles.

While rotating on its axis the zoospore moves forward sometimes in a slightly spiral way but mostly without conspicuous spiraling. This forward motion is accompanied by a constant vertical movement, the spore as it goes forward intermittently swimming up toward the surface of the water and then down, etc.

The zoospores of *Vaucheria* are not very suitable for critical observation by darkfield on flagellar structure or activity. By this method of observation I have not been able to determine the directions of the active flagella or whether or not both flagella of a pair behave similarly. After the zoospores are prepared by killing with osmium tetroxide fumes and then dried on the slide, most of the flagella along the perimeter of the spore are usually projecting outward, but in some cases the flagella on well over half of the visible surface are bent back roughly parallel with the surface.

Each flagellum of a pair arises from a densely staining granule. This is revealed in whole mount preparations of *V. geminata* (fig. 13) and in sectioned material of *V. pachyderma* (fig. 12), which show in most cases paired basal bodies. It cannot be determined with certainty from these preparations whether the basal bodies are extranuclear or whether the basal bodies are just within the nucleus and have stretched the nuclear membrane in migration to the surface membrane of the zoospore.

After dozens of preparations on the zoospores of many different collections of *V. geminata*—in one case the zoospores were from the very same group of filaments which later produced sex organs and the spermatozoids which are shown in figure 8—and after many preparations on the zoospores of one collection of *V. pachyderma*, the problem of the relative lengths and structure of the flagella of a pair is not completely solved.

That the two flagella of a pair are equal in length, as Dangeard observed, is often apparent in my preparations, but in many cases there is a distinct, though slight, difference in the lengths of the two (figs. 14–17), which one would expect in view of the heterokont spermatozoids. Due to inadequate techniques I have not been able to make measurements which might clarify the situation.

There is no clear difference in structure between the two flagella of a pair (figs. 14–17). The Löffler technique reveals no significant evidence of tinsels. Both flagella of a pair are structurally similar; i.e., of the same diameters, with smooth margins and pointed tips. Although there is some variation between flagella on the same spore as well as between spores, the pointed flagella are interpreted as being of the whip lash type. This is especially indicated when the lash ends are curved, bent, or coiled back with respect to the more rigid flagellum proper. In some cases, one flagellum was distinctly pointed or whip lash while the other of the pair was blunt-pointed.

The lack of consistency between flagellation of the spermatozoids and of the zoospores, shown by standard techniques, is certainly not expected. One must conclude that further study with different techniques is needed for the elucidation of this puzzling situation.

DISCUSSION

The motile, individual cell of the Chlorophyta has two equally long, apically inserted and posteriorly directed flagella both of which are of the whip lash type (fig. 20). In contrast to this, the motile cell of the Xanthophyceae has two unequally long and generally laterally attached flagella which extend in opposite directions; the anteriorly directed flagellum is of the tinsel type; the posteriorly directed flagellum is whip lash (fig. 19).

My observations have shown that the spermatozoids of *Vaucheria* are similar to the motile cells of the Xanthophyceae in that their two flagella are unequal, laterally inserted and oppositely directed, with the forward flagellum showing evidence of tinsels and the posteriorly directed one a whip lash flagellum. These two flagella, then, are structurally very different. It should be noted that in the case of *Vaucheria* the anteriorly directed flagellum is the shorter of the two; whereas, with the Xanthophyceae the reverse is typical.

Although P. Dangeard (1946) states that he finds on the compound zoospore of *Vaucheria* that the two flagella of a pair are quite equal in length, my preparations are considered to show that this is not definitely the case. It was surprising, though, and probably quite significant, to find that the two flagella of the zoospore are apparently of the same structure.

One must make a clear distinction between the two types of motile structures in *Vaucheria*. The uninucleate, biflagellate spermatozoid stands markedly in contrast to the multinucleate and multiflagellate zoospore. The zoospore, or synzoospore, is generally considered to be a compound structure consisting of a number of biflagellate swarmers which do not become delimited and fail to separate. The single-celled, colorless, and sometimes amoeboid spermatozoid may be considered the more primitive expression and of greater systematic significance than the synzoospore.

It is clear, then, as shown by flagellation, that the motile individual cell of *Vaucheria* indicates a closer affinity to the Xanthophyceae than to the Chlorophyta. This corroborates the diagnoses of the genus by Bohlin (1901) and Blackman and Tansley (1902), the cytological studies of Chadefaud (1945), and the pigment analyses of Strain (1948). On the other hand, the lack of consistency between flagellation of the spermatozoid and zoospore points to the theory that *Vaucheria* occupies a somewhat isolated systematic position.

There is a striking resemblance between the flagellated unicells of *Vaucheria* (fig. 18) and *Saprolegnia* (fig. 21), a member of the biflagellate series of the Phycomycetes. The *Saprolegnia*-type zoospore has two laterally inserted, oppositely directed, and structurally different flagella, and, as is also true for *Vaucheria*, in many cases the tinsel flagellum is slightly shorter. It has frequently been supposed that the Saprolegniaceae are closely related to or derived from the Siphonales, especially from a *Vaucheria*-like alga. Couch (1941) and Bessey (1942) state that if a study of the motile cells of the Higher Siphonales were to reveal the two types of flagella which are found in the Saprolegniaceae, one would have more reason for believing that the two groups are closely related. Longest (1946) found that flagellation of the male planogametes in *Codium*

decorticatum is typical for the green algae and on the basis of this concluded that this member of the Siphonales does not show kinship to the Phycomycetes. The present study of *Vaucheria*, which is apparently Xanthophycean and not a member of the Siphonales, shows that the motile individual cell of this alga and of the Saprolegniaceae are certainly very similar. Now that there is evidence on the basis of flagellation, modern systematic concepts of the Phycomycetes would logically permit, though certainly not require, a derivation of at least some of the Phycomycetes from a *Vaucheria*-like ancestor.

SUMMARY

(1) A study has been made on the reproductive structures of *Vaucheria* with particular stress on flagellation of the motile cells, and the significance of this study with respect to the relationships of *Vaucheria* to other algae and to the Phycomycetes is discussed.

(2) In the course of collecting and handling material it has been found that when *Vaucheria aversa*, *V. pachyderma*, *V. borealis* and *V. sessilis* grow terrestrially, their sexual organs hang down, which is not the case with the species of the *geminata* group. Also, the vegetative filaments apparently extend themselves in a spiraling way.

(3) The spermatozoids are distinctly heterokont, their two laterally inserted and oppositely directed flagella differing both in length and in structure. There is evidence that the shorter, anteriorly directed flagellum is of the tinsel type; the longer, posteriorly directed one is a whip lash flagellum. It is apparent from lightly stained spermatozoids that about one-half of the volume is occupied by the nucleus and that the basal granules from which the flagella arise are subapical or lateral.

(4) The pairs of flagella on the compound zoospore are equal or slightly unequal in length. There is no significant evidence of tinsels on either flagellum of a pair; both are pointed or whip lash. Each flagellum is attached to a basal body just within the outer membrane of the zoospore.

I am especially grateful to Dr. J. N. Couch for his guidance during the course and in the preparation of this study. I would also like to thank Mrs. Alma H. Beers and Dr. J. E. Adams for aid in preparing the manuscript, and Miss E. K. Goldie-Smith for cultures of *Saprolegnia*.

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EXPLANATION OF PLATES

Drawings made with the aid of a Spencer camera lucida. Photographs taken with an E. Leitz "Makam" attachment.

PLATE 3

FIG. 1. Photomicrograph of terrestrial *Vaucheria aversa*. The antheridium and oogonium hang down toward the earth. The wanderplasm (WP) is here just leaving the oogonium. (× 120)

FIG. 2. Photomicrograph of terrestrial *Vaucheria pachyderma*. The sexual organs here are also directed toward the earth. The wanderplasm (WP) has passed out of the oogonium and into the subtending filament. This photograph was taken about 30 min. after the stage comparable to that shown in fig. 1, or about 3 hrs. after the initiation of migration. (× 200)

FIG. 3. Photomicrograph of spermatozoids of *Vaucheria pachyderma* stained by Löffler technique. (× 1116)

FIG. 4. Spermatozoids of *Vaucheria pachyderma* stained by Löffler technique. a, × 4067; b, × 2440. Note tinsels on anterior flagellum and whip lash on posterior flagellum.

FIG. 5. Spermatozoids of *Vaucheria sessilis* stained by Löffler technique. a, × 2440; b, × 4067. Note irregular edges of anterior flagellum.

FIG. 6. Spermatozoids of *Vaucheria aversa* stained deeply with crystal violet. (× 2480)

FIG. 7. Spermatozoids of *Vaucheria sessilis* stained lightly with crystal violet, showing internal structure. (× 2480)

FIG. 8. Spermatozoids of *Vaucheria geminata* stained deeply with crystal violet. (× 2480)

PLATE 4

FIG. 9. Photomicrograph of *Vaucheria geminata* showing the tips of filaments producing zoospores. The sporangium at upper right nearly mature. The zoospore at lower left spinning about its longitudinal axis during its escape through the apical pore of the sporangium. Sometimes the zoospore spins out with a gyrating, spiral movement as shown by the middle figure. These spores were killed and fixed by mild heat for photographing. (× 100)

FIG. 10. Photomicrograph of *Vaucheria* sp. showing the large anterior vacuole of the actively swimming, ovoid zoospore (3rd from right). To the left is an encysted zoospore. The other two encysted zoospores are germinating. (× 100)

FIG. 11. The filament producing the zoosporangium has proliferated through an old empty sporangium. (*Vaucheria geminata*—not camera lucida.) (c. × 50)

FIG. 12. Portions of zoospores of *Vaucheria pachyderma* sectioned at 4 or 6 μ. a, stained with crystal violet; b, stained with hematoxylin. f, portion of flagellum; o, outer membrane; n, nucleus; c, chloroplast; v, large anterior vacuole. (× 2480)

FIG. 13. Portion of the rim of zoospore of *Vaucheria geminata* which had been killed with osmium tetroxide fumes, dried on slide and then stained lightly with crystal violet. The basal bodies and nuclei were not visible until a year after preparation, presumably as a result of differential fading. (× 2480)

FIG. 14. Pairs of flagella of *Vaucheria pachyderma* which had pulled away from spore during drying. Löffler stain. (X 2480)

FIG. 15. Portion of surface of zoospore of *Vaucheria geminata* stained by Löffler technique. (× 2480)

FIG. 16. Pair of flagella of *Vaucheria* sp. which had pulled away from spore during drying. Stained deeply with crystal violet. (× 2480)

FIG. 17. Portion of surface of zoospore of *Vaucheria pachyderma* stained by Löffler technique. (× 4140)

PLATE 5

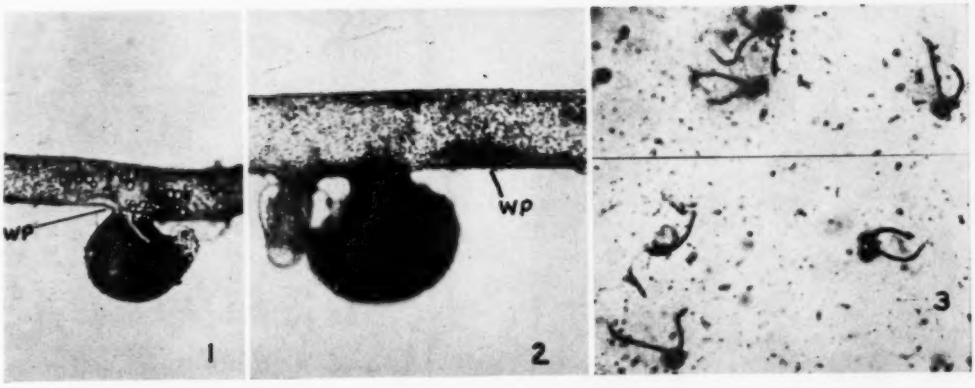
FIG. 18. Spermatozoids of *Vaucheria pachyderma* stained by Löffler technique. Photomicrograph × 2425. Drawings × 2440.

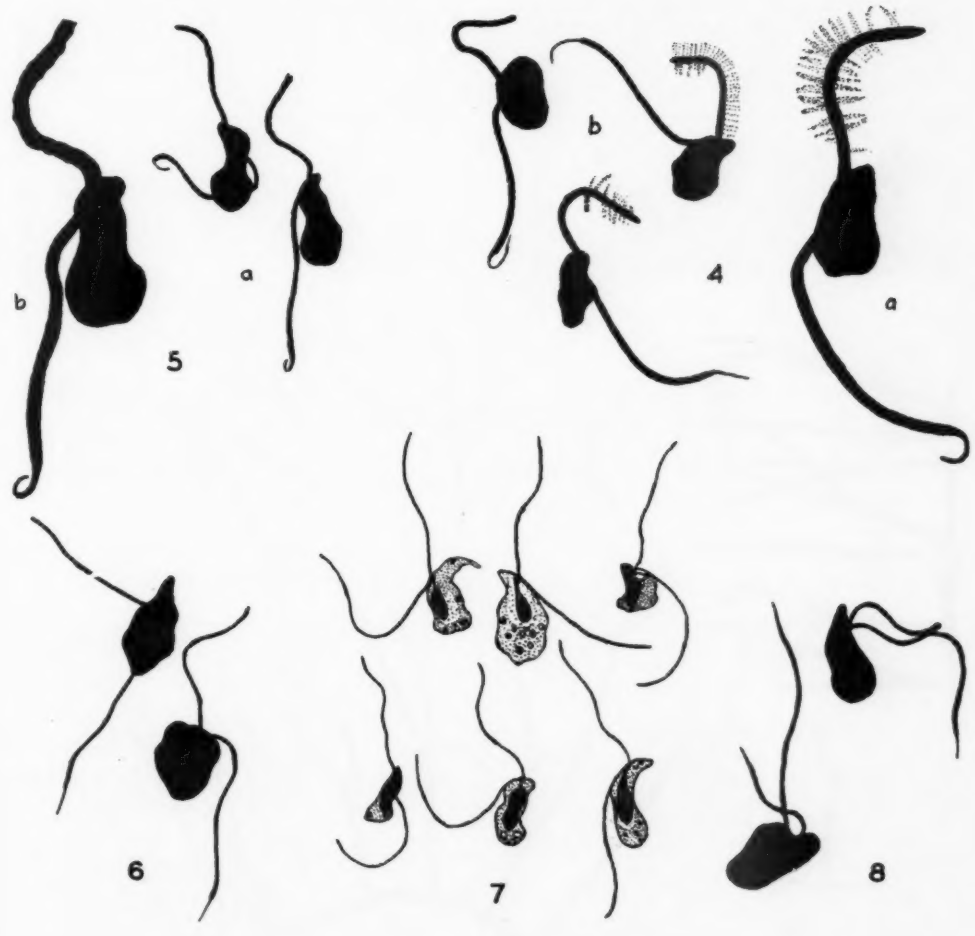
FIG. 19. Characteristic motile cell of the Xanthophyceae: zoogametes of *Botrydium granulatum* stained by Löffler technique. Photomicrograph × 1116. Drawing × 1660. The preparation photographed showed no whip lashes on the shorter flagellum.

FIG. 20. Characteristic motile cell of the Chlorophyta: zoogametes of *Chlamydomonas* sp. Photomicrograph × 1116. Drawings × 1660. a. Stained by Löffler technique. Note that the whip lashes show up merely as pointed ends when these flagella are stained by the Löffler method. b. Stained with crystal violet.

FIG. 21. Characteristic motile cell of the biflagellate series of Phycomycetes: zoospores of *Saprolegnia diclina* stained by Löffler technique. Photomicrograph × 1164. Drawing × 1660.

PLATE 3





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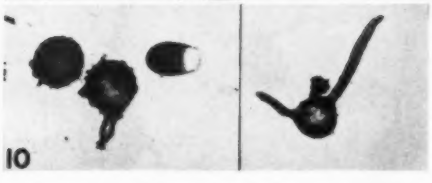
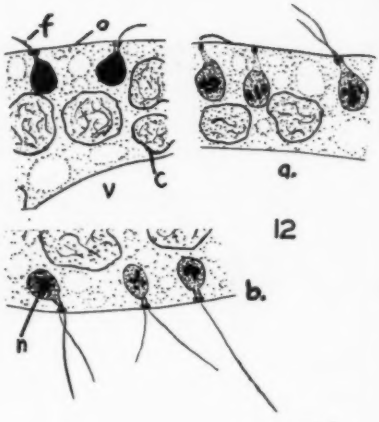
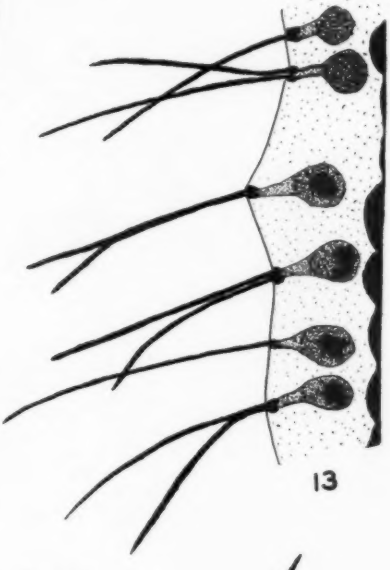






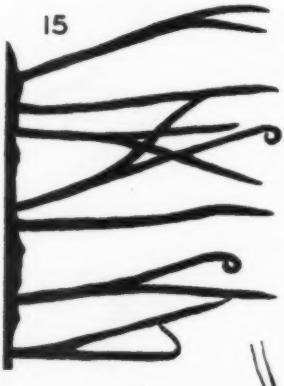
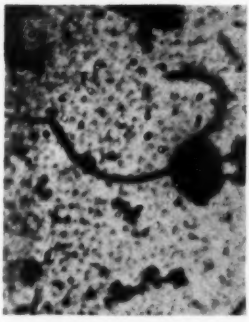
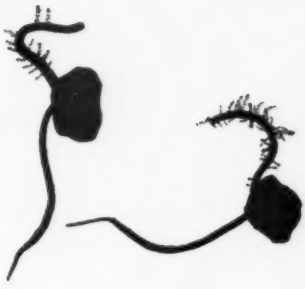


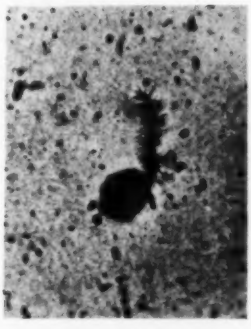
PLATE 5



VAUCHERIA

18





BOTRYDIUM

19

CHLAMYDOMONAS

20

SAPROLEGNIA

21

THE BIOTA OF THE REEFS OFF THE CAROLINAS

By A. S. Pearse

Duke University, Durham, North Carolina

AND

Louis G. Williams

Furman University, Greenville, South Carolina

Five Figures

Introduction

The reefs that occur off the coast of North Carolina and South Carolina are of various extents and are found at various depths. Some are close inshore, others are far out and in the western edge of the Gulf Stream. They are called "Black Rocks" by fishermen because they furnish fine fishing grounds for the blackfish, *Centropristis striatus* (L.).

These reefs have been generally believed to be composed of coral formations, but those investigated prove to have a foundation of Trent Marl, Lower Miocene (Figs. 1, 2), according to Dr. David Nicol, to whom specimens of this fossil material were sent. The dominant fossil was a pelecypod, *Venus gardneri* Kellum. These fossil rocks are overgrown in many places by calcareous tubes of living and sessile snails and tubicolous annelids (Figs. 3, 4). These substrata support many animals (Fig. 5) and an abundant growth of algae, in which encrusting forms dominate. Thorp (1935) states that the calcareous shallow water deposits of Florida and the Bahamas are made by organisms which contribute quantities in the following order: coralline algae, molluscs (*Vermetus*, etc.), Foraminifera, madreporian corals, alcyonarian corals, crustacean fragments, and Bryozoa. In Florida he says "worm rock" consists largely of *Vermetus* shells.

The junior author dived and collected from the rocks off New River Inlet, North Carolina, during the summers of 1947 and 1948. He also collected from these rocks at depths of 12 to 55 feet from June 10 to August 20, 1949. In all, ten diving trips during 1949 were made to these reefs. On August 16 and 17 two additional excursions were made to reefs off the mouth of Little River, South Carolina.

Thanks are due to the United States Fish and Wildlife Service for furnishing quarters for living and research at their station at Beaufort, North Carolina, where Messrs. Clinton Atkinson and Vance Fulford did everything possible to further the work. The Duke University Marine Laboratory also loaned diving equipment, buckets, and a fish trap. Private boats were chartered for the diving expeditions. Captain Martin Fulcher rendered excellent service for the trips from New River Inlet.

In making collections the junior author dived down to the reefs and brought up reef fragments with their plants and animals. In many cases it was necessary to use a crowbar to wedge organisms free that were encrusted to the reef. The

Poecilosclerina

Microciona prolifera (Ellis & Solander), S, N, BE.
Thalysurypon carteri (Topsent), D, F, Nw.
Thalysurypon ?foliacea (Topsent), D, S, Nw.
Myrmekioderma n. sp., D, Nw.

Halichondrina

PLATYHELMINTHES

Polycladida

Dr. Libbie Hyman identified five specimens as:
Coronadena mutabilis (Verrill), 8, NCS, BE.

Nemertea

Nemerteans were identified by Dr. [illegible]

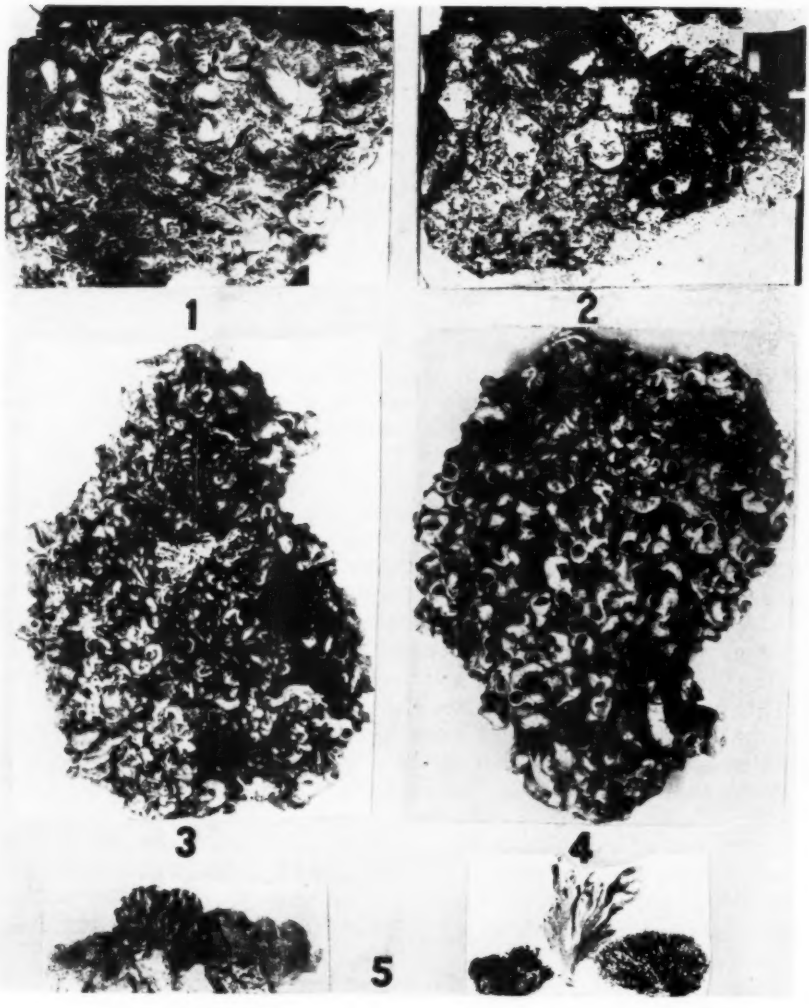


FIG. 1. Trent Marl that shows shells of *Venus gardneri*.
FIG. 2. Another view of Trent Marl that is somewhat more metamorphosed.
FIG. 3. Upper view of a piece of recent rock from a reef. The shells of sessile snails and worms are shown.
FIG. 4. Lower side of same rock shown in Figure 3. The snail shells are larger.
FIG. 5. Some sponges from the reefs.

organisms were brought up in gunny sacks. The catch was dumped into galvanized tubs and immediately sorted into pails of fresh sea water. Some small

specimens were preserved at once; the larger plants and animals were taken back to the dock and looked over in shallow enameled pans. Specimens were preserved for further study. The water in pails was finally poured through a sieve and the small plants and animals were thus secured.

ANIMALS OF THE BLACK ROCKS

BY A. S. PEARSE

The animals collected on the reefs were usually sent to specialists for identification and credit is given, following each group heading, to those who were kind enough to render such service. The works of these is greatly appreciated. Only macroscopic animals were collected or identified in the field. There are, therefore, no records of protozoans, larval crustaceans, and other microscopic animals. The following annotated lists doubtless do not include all the animals that were present on or in the rocks, but probably contain most of the common types. The surface of the rocks was rugged, full of fissures, cliffs, ledges, and crevices. Symbols are used in the annotated lists with the following meanings:

Symbols	*Description of symbols*
S	Shallow, 15 to 20 ft.
D	Deep, 40 to 55 ft.
SD	Animals taken both in shallow and deep waters.
BE	Beaufort estuary
F	From Florida south
N	North of North Carolina
NCS	From North Carolina southward

Symbols	*Description of Symbols*
Nw	New record for North Carolina
SCS	From South Carolina southward
MdS	From Maryland southward.
NS	Maine to Florida and West Indies along the Atlantic Coast
NYS	From New York southward
VaS	From Virginia southward
WC	West Coast of the United States

PORIFERA

(Fig. 5)

Dr. M. W. de Laubenfels, Oregon State College, identified 25 species of sponges.

Desmospongiae

Keratosa

Spongia graminea Hyatt, S, NCS, Nw.
Phyllospongia lanuga de Laubenfels, D, F, Nw.
Ircinia fasciculata (Pallas), SD, NS, Nw.
Ircinia campana (Lamarck), S, F, Nw.
Dysidea, sp., D, NCS, Nw.

Haplocerina

Haliclona ?coerulescens (Topsent), S, F, Nw.
Haliclona oculata (Linné), S, N, Nw.

Poecilosclerina

Microciona prolifera (Ellis & Solander), S, N, BE.
Thalyseurypon carteri (Topsent), D, F, Nw.
Thalyseurypon ?foliacea (Topsent), D, S, Nw.
Myrmekioderma n. sp., D, Nw.

Halichondrina

Higginsia coralloides Higgin, D, F.
Phakellia folium Schmidt, D, F, Nw.
Homaxinella sp., D, Nw.
Pseudaxinella sp., D, Nw.
Hymeniacidon heliophila (Parker), D, F, BE.
Ciocalapata n. sp.?, D, Nw.

Hadromerina

Pseudosuberites melanos de Laubenfels, D, F, Nw.
Cliona caribboea Carter, D, F, Nw.

Epipolasida

Dorypleres n. sp.?, D, Nw.

Choristida

Myriastra fibrosa (Schmidt), D, NS, Nw.
Geodia gibberosa Lamarck, D, F, Nw.
Craniella crania (Mueller), D, F, BE.
Cionchyra cavernosa (Lamarck), D, F, Nw.

Calcispongiae

Asconosa

Lucetta floridana (Haeckel), SD, F, Nw.

COELENTERATA

Hydrozoa

Sertularia gracilis Hincks, SD, NS.
Sertularia conica Allman, S, NS, BE.
Monostaechas quadridens McCrady, S, NS, BE.
Clytia johnstoni (Alder), S, NS, BE.
Eudendrium carneum Clarke, SD, NCS, BE.
Aglaophemia rigida Allman, SD, NS, BE.

Anthozoa

Astrangia astraeiformis Leidy, SD, NS, BE.
Cyclista leucolena (Agassiz), S, NS, BE.
Leptogorgonia virgulata (Lamarck), S, NCS, BE.
Gorgonia bipinnata Verrill, SD, NCS, BE.

PLATYHELMINTHES

Polycladida

Dr. Libbie Hyman identified five specimens as:
Coronadena mutabilis (Verrill), S, NCS, BE.

Nemertea

Nemerteans were identified by Dr. Wesley R. Coe.

Heteronemertea

Micrura leidyi (Verrill), S, N, BE.

Hoplonemertea

Oerstedia dorsalis (Abildgaard), S, N, BE.
Amphiporus ochraceus (Verrill), S, N, BE.
Amphiporus n. sp., S, Nw.
Proneurotes multioculatus Montgomery, S, N, Nw.

BRYOZOA

Bryozoans were identified by Dr. R. C. Osburn.

Cyclostomata

Crisia eburnea (L.), SD, NS.
Crisia elongata Milne-Edwards, D, F, Nw.
Crisulipora orientalis Canu & Bassler, S, F, Nw.

Ctenostomata

Amathia vidovici (Heller), S, NS.
Amathia convoluta Lamouroux, S, F, Nw.
Sundanella sibogae (Harmer), S, F, Nw.

Cleilostomata

Aetea anguina (L.), SD, NS.
Membranipora tenuis Desor, S, NS.
Canopeum commensale Kirkpatrick & Metzlaar, D, F, Nw.
Crassimarginatella leucocypha Marcus, SD, F, Nw.
Thalamoporella gothica var. *floridana* Osburn, S, F, Nw.
Caulibugula sp., S.
Cribrilina punctata (Hassall), SD, NS.
Hippothoa hyalina (L.), S, NS.
Schizoporella unicornis (Johnston), S, F, BE.
Schizoporella floridana Osburn, S, F, Nw.
Microporella ciliata (Pallas), D, NS.
Hippororina contracta (Walters), SD, NS.
Smittina trispinosa var. *spathulata* (Smitt), SD, F, Nw.
Adeona violacea (Johnston), D, F, Nw.

Hippaliosina rostrigera (Smith), SD, F, Nw.
Hippoporidra edax (Busk), D, F, Nw.
Holoporella magnifica Osburn, S, F, Nw.
Schizmopora dichotoma (Hincks), S, F, Nw.

Osburn says, "This is a strictly warm water bryozoan fauna, subtropical and similar to that of the Gulf of Mexico and the Caribbean. All but two of the species (*Crisia eburnea* and *Cribrilina punctata*) are recorded from the Gulf-West Indian region. *Hippothoa hyalina* apparently pays no attention to temperature and ranges from the high arctic to the tropics. *Crisia eburnea, Amathia vidovici, Aetea anguina, Membranipora tenuis, Cribrilina punctata, Microporella ciliata* and *Hippororina contracta* range northward to temperate waters as far as Cape Cod or further, and all but the two mentioned enter the tropics."

ANNELIDA

Polychaeta

Dr. Olga Hartman identified the polychaetes.

Polynoidae

Harmothoe aculeata Andrews, S, NCS, BE.
Lepidonotus variabilis Webster, D, VaS, BE.
Lepidonotus sublaevis Verrill B, N, BE.

Chrysoptelidae

Bhawania goodei Webster, D, NCS, Nw.

Phyllodocidae

Nereiphylla fragilis (Webster), D, VaS, BE.
Phyllodocid fragments, B.

Syllidae

Trypanosyllis zebra Grube, B, NCS, Nw.
Syllis sp. D?

Nereidae

Platynereis dumerilii (Audouin & M. Edwards), S, W, NCS, BE.
Ceratonereis tridentata (Webster), D, NJ to NC, BE.
Ceratonereis versipedata Ehlers, D, NCS, Nw.

Eunicidae

Eunice rubra Grube, B, VaS, BE.
Eunice filamentosa Grube, S, NCS, Nw.
Marphysa sanguinea (Montagu), B, NCS, BE.
Lysidice ninetta Audouin & M. Edwards, B, NCS, Nw.

Lumbrineridae

Lumbrineris latreilli Audouin & M. Edwards, S, NCS, Nw.
Lumbrineris inflata Moore, D, NCS, Nw.

Dorvilleidae

Dorvillea sociabilis (Webster), B, VaS, BE.

Orbiniidae

Naineris laevigata (Grube), S, W, WC, BE.

Cirratulidae

? Cirriformia sp., D, W, WC, BE.

Capitellidae

Dasybranchus ?lumbricoides (Grube), S, NCS, BE.

Maldanidae

Euclymene sp., S, NCS.

Sabellariidae

Sabellaria floridensis Hartman S, NCS, Nw.

Terebellidae

Amphitrides bruneo-comata (Ehlers), S, NCS, Nw.
Terebellid fragments, S.

Sabellidae

Sabella melanostigma (Schmarda), S, NCS, Nw.
Sabella microphthalma Verrill, B, N, BE.
Megalomma near *lobifera* (Ehlers), B, NCS, Nw.

Serpulidae

Eupomatus dianthus (Verrill), B, W, BE.
Vermiliopsis annulata (Schmarda), D, NCS, Nw.
Salmacina ?dysteri Huxley, B, N, BE.
Spirorbis sp., B.

In regard to the polychaetes Dr. Hartman says, "The polychaetes are largely those common to the West Indies and the Bermuda Islands; some species are known more widely along the eastern coast of the United States, from New England southward. The superfamily Eunicea is best represented, with 7 species and more than 100 specimens. Others to be expected in subtropical seas, such as the fire-worms (Amphinomidae) and phyllodocids, are nearly or quite absent; it is likely that their representatives, because of their larger size and stronger locomotive powers, escaped the snares employed in diving technics."

Hirudinea

Leeches were identified by Dr. Marvin C. Meyer.
Pontobdella (*Pontobdellina*) *macrothela* Schmarda, S, NCS, Nw.

Sipunculida

The writer identified a sipuncilid as
Siphonosoma cumanense (Keferstein), S, NCS.

ECHINODERMATA

Dr. Austin H. Clark identified the echinoderms, except the holothurians and *Echinaster*.

Asteroidea

Luidia alternata (Say), D, NCS.
Thyraster serpentarius (Müller & Troschel), SD, F, Nw.
Asterias forbesi (Desor), D, NS, BE.
Echinaster spinulosus Verrill was collected on a reef $1\frac{1}{2}$ miles east of the New River Inlet by Dr. Irene McCulloch on June 30, 1947, and identified by Elizabeth Deichmann. S. F.

Ophiuroidea

Amphiopholis gracillima (Stimpson), D, W, BE.
Ophiothrix angulata (Say), SD, NS, BE.
Ophioncreis reticulata (Say), SD, NCS, Nw.

Echinoidea

Arbacia punctulata (Lamarck), SD, NS, BE.
Lytechinus variegatus (Lamarck), SD, NCS, BE.
Clypeaster prostratus Ravenel, D, NCS.
Mellita quinquiesperforata (Leske), SD, NCS, BE.

Holothuroidea

Cucumaria pulcherrima (Ayers), SD, NYS, BE.
Thyone briareus (LeSueur), SD, NYS, BE.

MOLLUSCA

Dr. Harold A. Rehder identified 35 recent molluscs and Dr. David Nicol names two fossil (*) clams. The geographic ranges have been taken from Johnson (1934).

Pelecypoda

Nucula proxima Say, S, NS, BE.
Arca umbonata Lamarck, SD, NCS, Nw.
Acar reticulata Gmelin, S, NCS, Nw.
Pteria colymbus Röding, SD, NCS, BE.

Ostrea permollis Say, SD, NCS, Nw.
**Anomia onslowensis* Richards, SD.
Volsella americana Leach, SD, NCS, Nw.
Lithophaga bisulcata Orbigny, SD, NCS, BE.
This date shell bores into the reef.
Gregariella coralliophaga Gmelin, S, F, Nw.
Lyonsia beana Orbigny, SD, NS.
Chama macerophylla Gmelin, D, F, Nw.
Taras semiaspara Philippi, SD, NCS, Nw.
Chione grus Holmes, B, NCS, Nw.
**Venus gardneri* Kellum, SD.
Rupellaria typica Jones, SD, NCS, Nw.
Rupellaria cuneiformis Spengler, SD, NCS, Nw.
Cumingia tellinoides Conrad, S, NS, Nw.
Saxicava rugosa Linné, SD, W.
This clam bores into the reef.

Amphineura

Acanthochitona spiculosa Reeve, SD, F, Nw.

Gastropoda

Diodora cayenensis Lamarck, SD, NS.
Calliostoma euglyptum A. Adams, SD, NCS, Nw.
Turbo castaneus Gmelin, SD, NCS, BE.
Crepidula aculeata Gmelin, SD, NCS.
Crepidula fornicata Linné, SD, NS, BE.
Crepidula plana Say, D, NS, BE.
Vermicularia spirata Philippi, SD, NS, Nw.
This sessile snail is an important reef builder.
Hipponyx sp., D.
Simnia uniplicata Sowerby, S, NCS, BE.
Pseudoneptunea multangulata Philippi, SD, NCS, Nw.
Urosalpinx cinereus Say, S, NS, BE.
Favartia cellulosa Conrad, D, NCS, Nw.
Anachis avara Say, SD, NS, BE.
Aplus tinctus Conrad, SD, NCS, Nw.
Nassarius vibex Say, D, NS, BE.
Phos sp., D, Nw.
Fasciolaria distans Lamarck, S, NCS, BE.
Fasciolaria gigantea Kiener, S, NCS, Nw.
Marginella nivosa Hinds, SD, NS, Nw.

Cephalopoda

Octopus sp., S.
Three young octopi were collected on June 19.

Loligo pealei LeSueur D, NS, BE.
Reported off Little River, S. C., by fishermen.

ARTHROPODA

Pycnogonida

Sea "spiders" were identified by Dr. Joel W. Hedgpeth.
Anoplodactylus lentus Wilson, SD, N, BE.

Crustacea

Cirripedia

Calanus eburneus Gould, S, NS, BE.
Balanus amphitrite niveus Darwin, D, NS, BE.
Two sacculinids were taken on crabs. These were identified by Dr. Edward G. Reinhard as
Lenaeodiscus porcellanae Müller, D, F, Nw.
On *Petrolisthes galathinus* (Bosc).
Sacculina hirsuta Boschma, D, F, Nw.
On *Pilumnus dasypodus* Kingsley

Isopoda

Cirolana impressa Harger, D, N, Nw.
Sphaeroma quadridentatum Say, S, NS, BE.
Paracerceis caudata (Say), B, MdS.
Erichsionella attenuata (Harger), S, N, Nw.
Bopyro choprae Pearse, S, F, Nw.
From *Synalpheus fritzmulleri elongatus* Coutière and *S. minus* (Say), S.
Diplophryrus synalphei Pearse, S, NS, Nw.
From *Synalpheus fritzmulleri elongatus* Coutière, S, NCS.

Amphipoda

Amphipods were identified by C. R. Shoemaker.

Lysianopsis alba Holmes, S, N, BE.
Ampelisca schellenbergi Shoemaker, SD, F, WC, Nw.
Ampelisca sp., S.
Leucothoe spinicarpa (Abildg.), SD, NS, BE.
Colomastix pusilla Grube, S, F, Nw.
Stenothoe sp., S, Nw.
Batea catharinensis Müller, S, NS, Nw.
Elasmopus sp., SD.
Elasmopus rapax Costa, SD, NS.
Melita fresnelii (Audouin), SD, NCS, BE.
Maera sp., B.
Maera inaequipes (Costa), S, F, Nw.
Lembos smithi (Holmes), SD, N, BE.

Photis pugnator Shoemaker, S, F, Nw.
Eurystheus maculatus (Johnston), SD, F, Nw.
Eurystheus erythrophthalmus (Latreille), S, F, Nw.
Synamphithoe pelagica (M.-Edw.), S, NS, Nw.
Cerapus tubularis Say, SD, N, Nw.
Erichthonius brasiliensis Dana, S, NS.
Corophium simile Shoemaker, SD, NS, Nw.
Unciola laminosa Pearse, S, NS, Nw.
Caprella acutifrons Latreille, S, NS, BE.

Decapoda

Natantia

Peneidea

Decapods were identified by Dr. Fenner A. Chace, Jr.

Sicyonia laevigata Stimpson, D, NCS.
Crangon formosus (Gibbes), D, NCS, BE.
Crangon normanni (Kingsley), S, F.
Synalpheus fritzmulleri Coutière, S, F.
Synalpheus fritzmulleri elongatus Coutière, SD, NCS.
Synalpheus longicarpus (Herrick), D, SCS.
Synalpheus minus (Say), SD, NCS, BE.
Synalpheus townseni Coutière, D, NCS, BE.

Caridea

Latreutes parvulus (Stimpson), D, NCS.
Thor floridanus Kingsley, SD, NCS.

Reptantia

Anomura

Petrolisthes galathinus (Bosc), S, NCS, BE.
Porcellana soriata Say, SD, NCS, BE.
Periclimenes longicaudatus (Stimpson), D, NCS.
Periclimenes sp., D.
Periclimenaeus sp., S.
Processa sp., D.
Paguristes torgugae Schmitt, SD, NCS.
Pagurus near *bonairensis* Schmitt, B, F.
Pagurus sp., SD.
Paguridae spp.?, S.

Brachyura

Speloeophorus nodosus (Bell), D, NCS, BE.
Portunus sayi (Gibbes), S, W, BE.
Callinectes sapidus Rathburn, S, NS.

Callinectes sp., S.
Leptodius agassizii A. Milne-Edw., D, NCS, BE.
Pilumnus dasypodus Kingsley, SD, NCS.
Pilumnus floridanus Stimpson, S, NCS.
Pilumnus pannosus Rathbun, D, NCS.
Pilumnus sayi Rathbun D, NCS, BE.
Metoporhaphis calcarata (Say), D, NCS, BE.
Podochela riisei Stimpson, D, NCS, BE.
Epialtus dilatatus A. Milne-Edw., S, F, Nw.
Epialtus sp. near *sulcirostris* Stimpson, S, WC.
Epialtus sp. near *minimus* Lockington, S, WC.
Pelia mutica (Gibbes), S, NS, BE.
Mithrax (*Mithraculus*) *forceps* (A. Milne-Edw.), S, NCS, BE.
Mithrax (*Mithrax*) *pleuracanthus* Stimpson, D, NCS, BE.
Microphrys antillensis Rathbun, SD, NCS.

Stomatopoda

Gonadactylus oerstedi Hansen, D, NCS, BE.

CHORDATA

Tunicata

Ascidians were identified by Dr. Willard G. Van Name.
Amaroucium stellatum Verrill, SD, N.
Tridemnum savignii (Herdman), S, F, Nw.
Didemnum canididum lutarum Van Name, SD, NS, BE.
Didemnum (*Polysyncraton*) *amethestum* (Van Name), B, F, Nw.
Eudistoma carolinense Van Name, S, NCS, Nw.
Eudistoma capsulatum (Van Name), S, F.
Eudistoma hepaticum (Van Name), S, NCS.
Clavelina pieta (Verrill), D, NCS.
Clavelina picta (Verrill), S, SCS.
Ecteinascidia turbinata (Herdman), S, F.
Ascidia interrupta Heller, S, NCS.
Symplegma viride Herdman, S, F, Nw.
Polyandrocarpa (*Eusynstyela*) *tincta* Van Name, D, F, Nw.
Styela partita (Stimpson), SD, NS.
Styela plicata (LeSueur), SD, NCS, BE.
Distaplia bermudensis Van Name, D, F, Nw.
Molgula occidentalis Traustedt, D, NCS.
Molgula manhattensis (DeKay), S, N, BE.

Pisces

Information concerning fishes caught on the Black Rocks was furnished by Martin Fulcher at Sneads Ferry, North Carolina, and by the captain of a party boat at Little River, South Carolina. Some (*) were caught on the trips we took.

A few (†) were sent to the American Museum of Natural History, where they were identified by Dr. John T. Nichols. Those indicated by "S" were caught by trollers near the surface; those indicated by "D" were caught with hook and line on the reefs; those marked "T" we caught in traps on the bottom. The first letters after the names have the same meaning as indicated on page 135.

Selachii

Carcharias taurus Rafinesque, SD, NS, BE.
Sphyrna tiburo (L.), D, NS.
Sphyrna zygaena (L.), D, NS.
Raja eglanteria Lacépède, D, NS.
Dasyatis americana Hildebrand & Schroeder, D, NCS, BE.
Manta birostris (Walbaum), D, NCS.

Teleostomi

Galeichthys milberti (Cuvier & Valiencennes), D, NS.
Leptocephalus conger L., SD, NS.
Brevoortia tyrannus (Latrobe), S, NS.
Synodus faetens (L.), SD, NS, BE.
Strongylura marinus (Walbaum), S, NS, BE.
Mugil cephalus L., SD, NS.
Mugil curema Cuvier & Valiencennes, SD, NYS.
Scomber scombrus L., S, N.
Auxis thazard Lacépède, S, NS.
Scomberomorus maculatus Mitchill, S, NS.
Gymnosarda alleterata (Rafinesque), S, NS.
Sarda sarda (Bloch), S, NS.
**Decapterus punctatus* Agassiz, SD, NS.
Caranx hippos (L.), SD, NS.
Caranx crysos (Mitchill), SD, NS.
Vomer setapinnis (Mitchill), D, NS, BE.
Trachinotus carolinus (L.), S, NS.
* *Seriola dumerili* (Risso), S, F.
T *Seriola zonata* (Mitchill), S, NS.
Pomatomus saltatrix (L.), S, NS.
Coryphaena hippurus L., S, NS.
Prionotus tricanthus (Peck), D, NS, BE.
T* *Centropristes striatus* (L.), D, NS, BE.
Lutianus blackfordi Goode & Bean, D, NS.
T* *Orthopristis chrysopterus* (L.), D, NS, BE.
T* *Haemulon parra* (Desmarest), D, F.
Haemulon sciurus (Shaw), D, F.
* *Haemulon macrostomum* Günther, D. F.
Stenotomus aculeatus Cuvier & Valiencennes, D, NCS.

T* *Lagodon rhomboides* (L.), D, NS, BE.
Archosargus probatocephalus (Walbaum), D, NS, BE.
Archosargus unimaculatus (Bloch), D, F.
T* *Diplodus holbrooki* (Bean), D, NCS.
Cynoscion regalis (Block & Schneider), D, N, BE.
Sciaenops ocellatus (L.), D, NS, BE.
Leiostomus xanthurus Lacépède, D, NS, BE.
Micropogon undulatus (L.), D, NS, BE.
Menticirrhus saxatilis (Bloch & Schneider), D, NS, BE.
Progonias cromis L., D, N.
Tautoga onitis L., D, N.
T* *Balistes carolinensis* Gmelin, D, NYS.
T* *Spheroides maculatus* (Bloch & Schneider), D, NYS.
Lagocephalus laevigatus (L.), D, NCS.
Stephanolepis hispidus (L.), D, NS.
Prionotus carolinus (L.), D, NS, B
T* *Opsanus tau* (L.), D, W, BE.E.
† *Gobiesox strumosus* Cope, D, MdS, BE.
† *Hypsoblennius hentz* (LeSueur), D, MdS.
Eggs of this species were found attached to rocks.
† *Chasmodes bosquianus* (Lacépède), D, NYS.
Paralichthys lethostigmus Jordan & Gilbert, D, NYS, BE.
Achirus fasciatus Lacépède, D, NS.
Stymphrus plagiusa (L.), D, NCS.

Mammalia

Tursiops truncatus (Montague), SD, NS.
Delphinus delphis L., SD, NS.

Discussion

Agassiz (1880) and Thorp (1935) pointed out that the reefs north of Florida have been formed largely by the accumulation of shells, rather than by corals. Coralline seaweeds and foraminiferans have also contributed somewhat. On the Carolina coasts (Figs. 1, 2) additions to the reefs appear to be made at present, first by sessile snails (largely *Vermicularia spirata* Philippi). Some tubicolous annelids construct their own tubes. Two boring clams (*Saxicava rugosa* L., *Lithophaga bisulcata* Orbigny) also occupy holes in the calcareous layer formed by the snails and worms and in the underlying Trent Marl. Some sessile clams (*Ostrea*, *Mytilus*, etc.) add shells, particularly in crevices. Thus the reefs grow.

Temperature. There was little difference in temperature on the shallow and deep reefs. The winds and tides kept the shore waters stirred up. For example, on August 8 off Snead's Ferry at a depth of 45 ft. the temperature was 26° C. and the thermometer read the same at the surface. All the reefs studied were from a half mile to seven miles offshore so the warmer water in bays and estuaries had little influence on them. The maximum depths (55 ft.) were not great enough so that the cooler water in the deep sea was a factor.

Depth at which Species Occurred. Though the reefs studied were never more than 55 ft. deep, it was thought that a comparison of those collected from shallow reefs (15–20 ft.) with those from deeper (40–55 ft.) waters might show significant differences. Table 1 is an attempt at such a comparison. The species that occurred at greater depths showed a slightly greater tendency toward a more southern distribution: sponges 12-8, leeches 1, polychaetes 12-9, echinoderms 5-0, barnacles 2, decapods 15-12, and fishes 38-12. On the contrary some groups showed a greater tendency to correlate shallow water and southern distribution: polyclad 1, bryozoans 5-12, molluscs 6-9, isopods 1-4, amphipods

TABLE 1

The depths at which species of animals occurred. Shallow, 12–20 ft.; Deep, 40–55 ft.

	DEEP	SHALLOW	BOTH	TOTAL
Porifera	12	8	5	25
Hydrozoa		3	3	6
Anthozoa		2	2	4
Platyhelminthes		1		1
Nemertea		5		5
Bryozoa	5	12	7	24
Polycladida		1		1
Polychaeta	12	9	11	32
Hirudinea	1			1
Sipunculida		1		1
Echinodermata	5		8	13
Mollusca	6	9	23	38
Pycnogonida			1	1
Cirripedia	2	2		4
Isopoda	1	4	1	6
Amphipoda	1	12	10	23
Decapoda	15	12	9	36
Stomatopoda	1			1
Tunicata	4	9	5	18
Pisces	38	12	8	58
Mammalia			2	2
Total	103	102	95	300

1-12, and ascidians 4-9. Only a little more than a third showed any marked correlation between southern distribution and deep or shallow distribution. Most of the animals were wide-ranging on the coast or occurred in both shallow and deep water. Equal numbers of species were found in deep and shallow waters.

Latitudinal Range of Species. In Table 2 the general range of the species in various groups of animals as gleaned from literature is indicated. Some groups range mostly along the whole Atlantic coast (hydroids, nemerteans, and fishes); others occur in general from North Carolina southward (bryozoans, polyclads, polychaetes, echinoderms, molluscs, amphipods, decapods, stomatopods, and mammals); and two groups (sponges, tunicates) were found mostly from Florida southward into the West Indies. The isopods were rather evenly divided between

northern forms, those found from North Carolina south, and those distributed along the whole coast. On the whole most species (122:18) showed a tendency to have a distribution from North Carolina and Florida southward. And in some groups this was very pronounced (polychaetes, echinoderms, molluscs, decapods, sponges, bryozoans, tunicates, and amphipods). The fishes, barnacles, and coelenterates were the only groups that contained mostly wide-ranging species. Only eighteen species showed a tendency to range toward the north—bryozoans 2, polychaetes 4, pycnogonid 1, isopods 2, amphipods 3, tunicates 2, and fishes 4.

TABLE 2

Geographic ranges of species of animals collected. BE, Beaufort Estuary; F, Florida and south; N, generally north of North Carolina; NCS, North Carolina and south; Nw, new records for North Carolina; NS, widely distributed on the Atlantic coast of the United States

RANGE	NCS	N	F	NS	TOTAL	NW	BE
Porifera	3		20	2	25	20	3
Hydrozoa				6	6		5
Anthozoa	2			2	4		4
Platyhelminthes	1				1		1
Nemertea				5	5		3
Bryozoa	21	2		1	24	14	1
Polycladida	1				1		1
Polychaeta	20	4		4	32	12	15
Hirudinea	1				1	1	
Sipunculida	1				1		
Echinodermata	10		1	2	13	2	8
Mollusca	19		3	11	38	18	12
Pycnogonida		1			1		1
Cirripedia	2			2	4	2	2
Isopoda	2	2		2	6	4	1
Amphipoda	1	3	6	9	22	10	5
Decapoda	23		3	5	37	1	14
Stomatopoda	1				1		1
Tunicata	7	2	7	3	18	6	3
Pisces	6	4	5	43	58		19
Mammalia	2				2		1
Total	123	18	45	96	300	90	100

Food. The foods of reef animals are various. Sessile animals such as hydroids, corals, anemones, or bryozoans spread their tentacles and capture small living animals. Sponges, clams, and ascidians siphon water through their bodies and strain out plankters. A *Mytilus* may consume 100,000 lamellibranch larvae in 24 hours (Thorson, 1950). Some snails rasp algae off the rocks with their radulas, others bore holes through the shells of clams and devour the flesh of the animals inside. Crustaceans feed on living or dead animals, algae or plankton. Fishes are also predators, algae consumers, or plankton feeders. A blackfish consumes some

algae; a sheepshead may gnaw barnacles off a rock; a barracuda captures other fishes; a herring subsists on plankton.

Reproduction. Thorson (1950) has published an excellent summary of the reproduction and embryology of marine bottom animals. He points out that in general more eggs are wasted than in terrestrial or freshwater habitats and he differentiates three types of pelagic larvae: (1) lecithotrophic, from large eggs in small numbers; (2) planktrophic, with long life from numerous small eggs; and (3) planktotrophic with short life. There is no pelagic development in the deep sea. Most of it occurs in warm waters on tropical shelves.

Many of the shallow reef animals propagate and spread by means of pelagic larvae (sponges, coelenterates, bryozoans, annelids, crustaceans, many molluscs, echinoderms, and fishes). Many of them become attached (sponges, coelenterates, bryozoans, annelids, some molluscs) and some of these reproduce by budding (sponges, coelenterates, bryozoans, annelids) and often form great colonies. A few flatworms and crustaceans invade the bodies of other animals and live as parasites. Certain reef animals are sessile and commonly propagate by budding and produce pelagic larvae (sponges, coelenterates, bryozoans). Reef snails are generally creepers, but a few have become sessile and are important reef builders.

ALGAE OF THE BLACK ROCKS

BY LOUIS G. WILLIAMS

Except for citations of scattered records by various authors (Harvey, Farlow, etc.) no study of the marine algae of the middle Atlantic seacoast was done before that of Hoyt (1920). His studies were carried on intermittently, mostly during the summer months over a period of about six years. The area investigated ranged from Ocracoke, North Carolina, to Tybee, Georgia; but the greater part of the work was limited to the vicinity of Beaufort, North Carolina. From the total area, Hoyt reported 133 species and varieties. The habitats investigated were principally those of harbors, sounds and estuaries. Since along the Atlantic coast these areas have typically sandy or muddy bottoms and shorelines, they offer rather unfavorable natural habitats for marine algae. However, many of the forms reported by Hoyt were found on artificial substrata such as piers and especially rock walls and breakwaters or jetties, which offer favorable places for attachment of algae. Several were obtained also by dredging offshore rock reefs and others were collected on beaches where they had drifted ashore. After three collections from dredging trips on the reefs, Hoyt (1920) reported 47 species. Only four identified species were found in all three of his collections from the reef, while 14 were obtained in two collections, and 29 were found only once. He says, "This is probably an indication not so much of a seasonal distribution as of the abundance of the species occurring there and our ignorance of them. It is highly desirable that a thorough study of these reefs be made." Blomquist & Pyron (1943), following a storm that hit the Carolinas after sweeping through the Bahamas in August, 1940, reported a number of tropical species washed ashore, which were unreported from the Carolinas. Hoyt realized that species

only found adrift or on a beach cannot be strictly included in the local flora, since it is impossible to distinguish between those that come from reefs along the shore and those that are brought in from distant regions, but they have been enumerated by him as a part of the flora from the vicinity of Beaufort, North Carolina. In a report of marine algae new to Beaufort, Blomquist & Humm (1946) included nine species which were collected from sea buoys or were free-floating. Most of the species that have been reported as drifting or attached to sea buoys are known to be species that only grow attached. Inasmuch as these species have not been reported in an attached condition from the estuaries of

TABLE 3

Some general ecological and distributional data of species of marine algae collected on the submerged reefs lying off the coast of the Carolinas

SYMBOLS	DESCRIPTION OF SYMBOLS	NUMBERS	TOTALS
S	Shallow, to 20 ft.	29	
D	Deep, 40 to 55 ft.	26	
SD	Both shallow and deep	49	104
NCS	North Carolina and southward, tropical Western Atlantic Ocean including one or several of the regions as follows: Bermuda, Jamaica, Puerto Rico, the Virgin Islands, Caribbean Islands, Guadeloupe, Barbados, and northern Brazil.	56	
NS	North and south of North Carolina, widely distributed	29	
CO	Carolinas only	11	
	Distribution unknown	8	104
O	Oceanic species or varieties in our locality	64	
EO	Estuarine and oceanic in our locality	40	104
A	Abundant	14	
C	Common	22	
I	Infrequent	57	
R	Rare	11	104
N	Reported here for the first time from the Carolinas		23

the Carolinas, it is reasonable to assume that environmental factors in these inshore waters are unfavorable for their attachment and growth, and that environmental conditions, probably oceanic, do exist on the reefs and this has made possible the development of reef communities of oceanic species, for the most part, not to be found in the estuarine waters. This assumption is supported by recent findings at Cape Lookout. After a year-around intensive investigation of the marine algae of a jetty at Cape Lookout, the writer (1948), reported 60 new records of attached algae for North Carolina. The presence of species at Cape Lookout, which have not been reported from the near-by Beaufort estuary, may be explained also by the fact that environmental conditions there are nearly oceanic, since the cape extends out into the open ocean, while those at Beaufort

are estuarine. A natural outcropping of coquina rock at Fort Fisher supports an algal flora which is in several respects similar to that found at the beach end of the jetty at Cape Lookout.

The purpose of the present investigation was to secure direct information concerning the quality, quantity, and ecology of the attached plants of the reefs, which have been studied by previous investigation only by dredging or from algae washed ashore, and to attempt to arrange categories for the marine algae of these reefs into natural groups based on (1) depth (shallow, deep, or both); (2) effects of continental drainage (estuarine or oceanic); (3) north-south distribution (North Carolina and tropical, occurring both north and south of North Carolina, and reported in the Western Atlantic only from the Carolinas); (4) population data (abundant, common, frequent, or rare); (5) species newly reported to the Carolinas; and (6) species previously reported as found in the Carolinas, but reported here for the first time attached. The writer is indebted to Dr. W. R. Taylor, who identified or annotated a number of these species, to Dr. H. L. Blomquist for helpful criticisms and suggestions, and to Furman University for a Carnegie grant in aid. Symbols used in the systematic list are given in Table 3.

SCHIZOPHYTA

Hormogonales

Calothrix confervicola Born. and Flah., SD, NCS, O, R.
Determined by Dr. Francis Drouet.

CHLOROPHYTA

Ulotrichales

Entocladia viridis Reinke, SD, NS, EO, C.

Ulvella lens Crouan, SD, CO, O, A.

Cladophorales

Chaetomorpha brachygona Harv., SD, NCS, EO, I.

Chaetomorpha Linum (Müll.) Kütz., S, NS, O, I.

Cladophora delicatula Mont., SD, NCS, O, I.

Cladophora pellucida (Huds.) Kütz. (*C. prolifera* (Roth) Kütz.), SD, CO, O, A.

This identification was verified by Dr. Harry K. Phinney. That this species has not been previously found by dredging is not surprising since it occurs only in sandy depressions, where it is firmly attached to rock. It is reported from North Carolina by Hoyt who found it washed ashore.

Siphonales

Avrainvillea Mazei Murray & Boddle (*A. longicaulis* (Kütz.) C. Ag., D, NCS, O, N, I.

Caulerpa prolifera (Forssk.) Lamour., D, NCS, O, I.

Codium decorticatum (Woodw.) Howe, SD, NCS, EO, C.

After studying dried, herbarium specimens, and pickled material from Europe labeled *Codium elongatum* C. Ag., 1822, it now appears that this

material is the same as that from North Carolina which has been passing under the name of *C. decorticatum* (Woodw.) Howe, 1911. However, since only pictures of the type material of *C. elongatum* have been examined, it seems best, at present, to use the later described *C. decorticatum*. After several years of study of the size of utricles in material of *C. decorticatum* from North Carolina it is apparent that the size of the utricles varies directly with the age and growth of the thallus, since the older utricles grow as the thallus grows. Some material of this species from the offshore reefs has the largest utricles encountered in North Carolina waters. From inshore waters it is not unusual to find material with utricles measuring 575μ in diameter, while on that from the reefs, collected in August, they may become as much as 700μ in diameter. *C. magnum* Dawson, 1950, has utricles that fall within this range and have other features similar to those of North Carolina material of *C. decorticatum*. Therefore, it is the opinion of the present writer that *C. magnum* Dawson, 1950, must be reduced to a synonym of *C. decorticatum*.

Codium isthmocladum Vickers, SD, NCS, O, I.

Derbesia vaucheriaeformis (Harv.) J. Ag., SD, NS, O, I.

Udotea conglutinata (Soland.) Lamour., D, NCS, O, I.

Udotea flabellum (Ell. et Soland.) Lamour., D, NCS, O, N, R.

Siphonocladiales

Valonia utricularis (Roth) C. Ag., D, NCS, O, R.

PHAEOPHYTA

Ectocarpales

Ectocarpus elachistaeformis Heydr., S, NCS, O, R.

Ectocarpus Mitchellae Harv., SD, NS, EO, I.

Ectocarpus terminalis Kütz., SD, NS, O, R.

Lithoderma fatiscens Kuckuck, (L. ?fatiscens Aresch.), SD, NCS, O, C.

Sphacelariales

Sphacelaria furcigera Kütz., SD, NS, EO, I.

Sphacelaria tribuloides Menegh., SD, NCS, O, I.

Dictyotales

Dictyopteris polypodioides (Desf.) Lamour., SD, CO, O, A.

Dictyopteris serrata (Aresch.) Hoyt, D, CO, O, A.

Dictyota cervicornis Kütz., S, NCS, EO, N, I.

This species was identified by Dr. W. R. Taylor. It is infrequent on the shallow reefs, but common on the jetties at Shackleford in the estuary.

Dictyota dichotoma (Huds.) Lamour., SD, NCS, EO, C.

Dictyota sp. SD, O, N, I.

Padina Vickersiae Hoyt, S, NCS, EO, R.

Typically an estuarine plant. Encountered once on shallow reef near New River Inlet.

Pocockiella variegata (Lamour.) Papenf. [*Zonaria variegata* (Lamour.) C. Ag.], D, NCS, O, C.

Since this alga does not occur in places accessible to dredging it has only been found previously washed ashore. It grows on the sides of fissures, and near the base of depressions.

Spatoglossum Schroederi (Mertens) J. Ag., SD, NCS, O, C.

Zonaria Tournefortii (Lamour.) Mont., D, CO, O, A.

Chordariales

Myrionema vulgare Thuret, SD, NS, EO, I.

Punctariales

Colpomenia sinuosa (Roth) Derb. and Sol., D, NCS, O, I.

Rosenvingea intricata (J. Ag.) Børg., D, NCS, N, O, I.

Fucales

Sargassum Filipendula C. Ag., S, EO, NS, I.

This species was encountered only near the mouth of New River Inlet, and is predominantly an estuarine plant.

Sargassum Filipendula C. Ag. var. *Montagnei* (Bailey) Collins & Herv., SD, NS, O, A.

Further investigation may prove that the last two listed entities are ecologic and not genetic.

Sargassum sp., D, O, I, N.

RHODOPHYTA

Bangiales

Erythrocladia subintegra Rosenv., SD, NCS, O, C.

Erythrocladia vagabunda Howe and Hoyt, SD, CO, O, I.

Nemalionales

Acrochaetium Hoytii Collins, SD, CO, EO, C.

Acrochaetium virgatulum (Harv.) Born., SD, NS, EO, I.

Acrochaetium robustum Børg., D, NCS, O, N, I.

Acrochaetium (*Rhodochorton*) sp., D, O, I, N.

Gelidiales

Gelidium coerulescens Kütz., SD, NCS, O, I.

Dr. W. R. Taylor (*in lit.*) questions the use of this specific name since it is a New Caledonian type, and, therefore, doubtfully Atlantic in range. By use of type material of this species the Atlantic plants may be shown to belong to an undescribed species.

Gelidium sp., S, O, I.

Probably *G. corneum* (Huds.) Lamour., but the reef plants are so variable they may be variants of the common *G. crinale* (Turner) Lamour. There is still another entity on the reefs and at Cape Lookout jetty that may eventually prove to be a species of *Gelidiella*.

Cryptonemiales

Amphiroa brasiliana Decaisne, SD, CO, O, A.

The largest and most abundant of the calcareous algae of reefs investigated.

Amphiroa fragilissima (L.) Lamour., D, NCS, O, C.

Contarinia Magdae Weber-van Bosse, D, NCS, O, N, R.

Material that agrees with the description of this species is sometimes found among the calcareous crusts.

Corallina cubensis (Mont.) Kütz. emend Børg., D, NCS, O, I.

Corallina officinalis L., SD, NS, O, C.

The form of this species from Carolina waters indicates that it is distinct from the form common in New England.

Cryptonemia crenulata J. Ag., S, NCS, O, C.

From North Carolina this species has not been previously reported in an attached condition, but it has been found washed ashore. It escapes being taken in a dredge because it occurs only in the fissures of the reef.

Epilithon membranaceum (Esper) Heydrich

(*Melobesia membranacea* (Esper) Lamour.), SD, NCS, EO, A.

Goniolithon sp., SD, O, I.

An unknown species present on the jetty at Cape Lookout, and forming a hard crust on the reefs.

Grateloupia filicina (Wulf.) C. Ag., S, NS, EO, I.

Halymenia Agardhii De Toni, D, NCS, O, I.

Found in depressions, and broad fissures in deep water.

Halymenia floridana J. Ag., D, NCS, O, I.

Gradations can be found between young thalli that could pass for this species to larger and older thalli that meet the general features of *H. Floresia* (Clemente) C. Ag., all occurring together in the same rocky depressions. Both of these species have been reported from North Carolina.

Halymenia Gelinaria Collins and Howe, D, NCS, O, C.

Hildenbrandia prototypus Nardo, S, NS, O, N, I.

This species grows mingled with the crusts of other algae and was to be expected since it occurs both North and South of the Carolinas.

Jania adherens Lamour., SD, NCS, O, I.

Jania capillacea Harv., D, NCS, O, C.

Mostly on stipes of *Sargassum*.

Lithophyllum Corallinae (Crouan) Heyd., SD, NS, O, A.

Determined by Dr. W. R. Taylor.

* *Lithophyllum intermedium* Foslie, SD, NCS, O, A.

* *Lithothamnium mesomorphum* Foslie var. *ornatum* Foslie & Howe, SD, NCS, O, A.

* *Lithothamnium* sp., SD, O, A.

Melobesia Lejolisii Rasanoff

Fosliella Lejolisii (Rasanoff) Howe, SD, NS, EO, C.

Peyssonelia conchicola Picc. and Grunow, SD, NCS, O, C.

Peyssonelia polymorpha (Zanardini) Schmitz, SD, NCS, O, I.

* Determination tentative, since only specialists of this group can determine specific entities.

Peyssonelia rubra (Grev.) J. Ag., SD, NCS, O, N, I.

Material of this species agrees with the treatment by Weber-van Bosse in Børgesen 1915–20.

Gigartinales

Agardhiella tenera (J. Ag.) Schmitz, S, NS, EO, R.

This is a rare species on the reefs, but it is very common in the estuaries.

Gigartina acicularis (Wulf.) Lamour., S, NCS, EO, R.

Gracilaria foliifera (Forssk.) Børg., S, NS, EO, I.

Part of the material of this species from the reefs can pass for *G. Curtissiae* J. Ag. from Florida.

Gymnogongrus sp., SD, EO, N, I.

Thus far an entity has not been found that will include this species, although it has some features of *G. tenuid* J. Ag. It is a larger plant than the common plant of the estuaries, *G. Griffithsiae* (Turner) Martius. This species has also been found on the jetties at Shackleford in the Beaufort estuary.

Hypnea cornuta (Lamour.) J. Ag., SD, NCS, EO, N, I.

Determined by Dr. W. R. Taylor. This species is rare on the reefs, but is common in the Beaufort estuary where it has been confused with *H. musciformis*. Here reported for the first time from the Carolinas.

Hypnea musciformis (Wulf.) Lamour., S, NS, EO, I.

Meristotheca Duchassaingii C. Ag., SD, NCS, EO, I.

Platoma sp., D, O, N, R.

Three young blades were found that apparently belong to this genus. Dr. Taylor says they are too young to determine.

Wurdemania setacea Harv., SD, NCS, O, I.

Rhodymeniales

Botryocladia uvaria (Wulf.) Kylin, D, NCS, O, I.

Was found only in deep water growing in scattered clumps on flat shallow-buried rocks and worm tubes.

Champia parvula (C. Ag.) Harv., SD, NS, EO, I.

Champia parvula (C. Ag.) Harv. var. *prostrata* n. var., SD, O, N, I, CO.

Thallis caespitosis epiphyticis, parvis, 20–35 cm. altis, conspicuo compressis; majoribus ramis ca. quinquies latioribus quam crassioribus; conferte congesteque ramosis e basi adhaeso haptera multis cellis. Cystocarpiis ortis in margine compressi erecti thalli, sessile, vel stipite brevi, 35–50 μ diam., ostiole conspicuo ca. 8 μ in diam. vix rostrato.

Thalli cespitose epiphytic, small, 20–35 cm. high, conspicuously flattened, larger branches about 5 times as wide as thick, congestedly branched from the base which is attached by multicellular haptera. Cystocarps borne on margin of flattened erect thalli, sessile or short-stalked, 35–50 μ at broadest diameter of dome-shaped pericarp. Carpospores pyriform, about 5 μ long by 3 μ at broadest diameter; ostiole conspicuous, about 8 μ in diameter, scarcely rostrate. Mature tetrasporangia about

10 μ in diameter, usually produced within the short branches. Antheridia not found.

Epiphytic on *Amphiroa brasiliana* and *Cladophora pellucida;* epizoic on *Sertularia conica*. This variety may be readily distinguished from *Champia parvula* (C. Ag.) Harv., and from *Champia compressa* Harv. by its smallness and persistent epiphytic habit, and its flattening.

Chrysymenia Enteromorpha Harv., D, NCS, O, C.

Lomentaria Baileyana (Harv.) Farlow, S, NS, EO, I.

Rhodymenia Palmetta (Esper) Grev., S, NCS, O, C.

This species strongly resembles *Leptofauchea rhodymenioides* Taylor in its vegetative development and by having marginal cystocarps, but Dr. E. Y. Dawson, who compared this material with the type of *L. rhodymenioides*, reports that the inside structure of the cystocarp is sufficient to place it in the genus *Rhodymenia*.

Ceramiales

Antithamnion cruciatum (C. Ag.) Nägeli, SD, NCS, O, I.

Callithamnion byssoideum Arnott, S, NS, EO, I.

Ceramium fastigiatum Harv., S, NS, EO, I.

Ceramium transversale Collins & Herv., SD, NCS, EO, N, I.

Chondria dasyphylla (Woodw.) J. Ag., S, NS, EO, C.

Chondria floridana (Collins) Howe, SD, NCS, O, N, A.

Chondria littoralis Harv., S, NS, EO, I.

Chondria sedifolia Harv., S, NS, EO, I.

Chondria tenuissima (Gooden. & Woodw.) C. Ag., S, NS, EO, R.

Griffithsia tenuis C. Ag., D, NCS, N, O, I.

Hoyt (1920) reports fragments dredged from the reef which he assigned to this genus but not to a species. The material found during this investigation does not belong to *G. globulifera* Harv., which is common in the West Indies, but fits the description of *G. tenuis* as found in Børgesen (1915–20).

Grinnellia americana (C. Ag.) Harv., S, NS, EO, I.

Hypoglossum tenuifolium (Harv.) J. Ag. var. *caroliniana* n. var., SD, O, I, N, CO.

Thallis flaccidis, rare altioribus quam 1 cm., ramosis sparsis e costa et stipitibus, lamina longa ad 6 mm. lata ad 2 mm. acuta vel emarginata, costa prope apicem juvenem 3 ordinibus cellarum per faciem superam, multis cellis latis apice laminae ubi sessile vel stipite brevi. Costa in sectione transversa prope apicem 3 cellis marginatis in utro latere 2 cellis superpositis, cellis crescentibus numero in sectione transversa ad spicem laminae in stipitem, male crassa multis cellis.

Fronds flaccid, rarely over 1 cm. high, branching sparsely from midrib and stalks, lamina to 6 mm. long and 2 mm. wide, acute or emarginate, midrib near young tip composed of three rows of cells in surface view,

becoming many cells wide at base of lamina where it is sessile or merges into a short stalk. The cross section of the midrib near the apex contains 3 cells bordered on either side by 2 superimposed cells; the number of cells in the cross section increases toward the base of the lamina and into the stalk, becoming a thick, multicellular structure.

This variety may be distinguished from *Hypoglossum tenuifolium* (Harv.) J. Ag. by the number of cells in the midrib, the presence of stalks formed independently of blades, and its small habit. It is also very similar to *H. Woodwardii* Kütz. of Europe, and except for attenuations might be *H. attenuatum* Gardner of the Pacific. Figured by the writer (1948) as *H. tenuifolium* (Harv.) J. Ag.

Laurencia scoparia J. Ag., S, NCS, EO, N, I.

Determined by Dr. W. R. Taylor. Not a typical reef plant. Found on portion of reef in mouth of New River Inlet, and uncommon on jetties at Shackleford in the Beaufort estuary.

Lophocladia trichoclados (Mert.) Schmitz, D, NCS, N, O, I.

Material answering the description and drawings, and similar to herbarium material of this species was found epiphytic on coarse algae of the deep reefs.

Nitophyllum medium Hoyt, S, CO, O, C.

Nitophyllum Wilkinsoniae Collins & Herv., S, NCS, O, N, C.

The last two named species are apparently the same species. Both were described in 1917, the former from North Carolina and the latter from Bermuda. However, there are two forms of Hoyt's species on the reefs. The spring and early summer form fits Hoyt's description, and the summer form belongs to the species by Collins and Hervey, and resembles P. B. -A., No. 2037.

Pleonosporium Borreri (Smith) Nägeli, S, NS, O, N, I.

Polysiphonia denudata (Dillwyn) Kütz., S, NS, EO, I.

Polysiphonia sphaerocarpa Børg., D, N, NCS, O, I.

This identification has been verified by Dr. G. J. Hollenberg. This alga is an epiphyte on stipes of *Sargassum*.

Spermothamnion investiens (Crouan) Vick., SD, NCS, EO, C.

Spermothamnion Turneri (Mert.) Aresch., SD, NS, EO, I.

Spyridia aculeata (Schimp) Kütz., S, NCS, EO, C.

Some of the material of this species approaches what has been called *S. clavata* Kütz.

DISCUSSION

While this investigation is primarily concerned with reef organisms, for purposes of evaluation and comparison a general description of the estuarine habitats in the Carolinas is given here, followed by a general treatment of marine ecology in light of recent findings. A number of ecologically different habitats occur within the inshore waters where some of the physical factors are subject to

fluctuations associated with fresh water drainage. Marine species show best development on jetties in parts of the estuaries where there is least mixture with fresh water and its contaminators in solution and suspension. The jetties at Shackleford, near Beaufort, are thus situated so that during the outgoing tide the salinity and transparency, as measured by standard methods, are more oceanic than those taken in the middle of the Beaufort inlet. A wide difference is noted during periods of freshets. In the Carolinas marine habitats and their algal communities may be classified into the five general categories as follows: (1) Brackish water that is not more than half sea water most of the time. Mostly salt marshes. Common species are *Herposiphonia tenella* (C. Ag.) Ambronn, *Caloglossa Leprieurii* (Mont.) J. Ag., and *Bostrychia radicans* (Mont.) Mont. (2) Estuarine water seldom less than 16 or over 28 parts per thousand salinity. Mostly mud flats. Common species are *Ulva Lactuca* L. and *Gracilaria confervoides* (L.) Grev. (3) Estuarine water seldom less than 25 or over 32 parts per thousand salinity. Sandy bottoms, stone jetties, and exposed peaty deposits. Characteristic species *Sargassum Filipendula* C. Ag., *Codium decorticatum* (Woodw.) Howe, *Hypnea musciformis* (Wulf.) Lamour., *Gelidium pusillum* (Stackh.) Le Jolis, *Chondria* spp. (4) Oceanic salinities, exposed rocky coast, as jetty at Cape Lookout and coquina outcrop at Fort Fisher, North Carolina. Common species are *Ulva fasciata* Delile, *Enteromorpha flexuosa* (Wulf.) J. Ag., *Gelidium crinale* (Turner) Lamour. (5) Oceanic salinities, offshore coastal submerged reefs (and the distal ocean end of Cape Lookout jetty), 12 to 65 ft. and more deep. Common species are *Pocockiella variegata* (Lamour.) Papenf., *Spatoglossum Schroederi* (Mert.) J. Ag., *Sargassum Filipendula* C. Ag. var. *Montagnei* (Bailey) Collins and Hervey, *Dictyopteris serrata* (Aresch.) Hoyt, *Cladophora pellucida* (Huds.) Kütz., and *Caulerpa prolifera* (Forssk.) Lamour.

The first four of these general habitats are pretty well known, but the offshore coastal reefs are only known from three dredging collections by Hoyt (1920). Because this study has involved repeated observations and collections by diving on to the reefs for first hand information at several different localities, it is possible to discuss certain phases of the algal ecology in some detail. While no special study was made of the distribution of species in relation to specific habitats, some impressions regarding this were gathered from general observations while collecting. The habitats may be generally classified into (1) those that pertained to rock structure (Trent Marl) extending several feet above the sandy sea floor, and (2) those about the general level of the sandy sea bottom. The reef varies greatly in relief. The surface is generally uneven and in many places broken or deeply fissured. Where the Trent Marl extends several feet above the general level of the sandy sea floor and away from the influence of shifting sand and silt associated with the bottom, encrusting algae seem to dominate. However, in this situation encrusting algae are not the most bulky or the most abundant (large brown algae lead in these respects) but these dominate in many places on the reefs because their crusts apparently exclude the development of holdfasts of other larger algae. There is no evidence that they have contributed materially to the Trent Marl as calcareous reef builders, but

in some of the deep sand pockets of the shallow reefs and in quieter water at 45 to 55 ft. sand has been loosely compacted by accumulated coralline and other calcareous deposits from algae and shells of mollusks. This is especially the situation where the compacted mantle has formed over the smooth, flat fossil Trent Marl of some of the deep reefs. Calcareous species were found growing over one another, which results in the death of the organisms so covered. Encrusting algae do not hold a static dominance, however, since they die and the dead crust furnishes attachment, for a time, to other algae and sessile animals. The crust is formed by species of *Peyssonelia*, *Lithophyllum*, *Lithothamnium*, and *Lithoderma*. From the crevices with a shallow covering of sand *Cladophora pellucida*, *Corallina officinalis*, *Amphiroa brasiliana*, *Amphiroa fragilissima*, and *Pockiella variegata* abound. In the deep protected fissures *Nitophyllum*, *Rhodymenia*, *Colpomenia*, *Halymenia*, *Chrysymenia*, *Cryptonemia*, *Grinnellia*, and *Caulerpa* together with hydroids, brilliantly colored sponges, and ascidians form beautiful marine gardens.

In massive areas associated with the projected reefs the Trent Marl has been eroded down to form a flat sea floor made level by sand which fills the depressions and crevices. Here large brown algae abound, such as *Sargassum Filipendula* var. *Montagnei*, *Sargassum* sp., *Dictyopteris polypodioides*, *Dictyopteris serrata*, and *Spatoglossum Schroederi*.

The present investigation clearly shows that the reefs support a summer flora that is tropical. However, the absence of species known to be common in other parts of the tropical Western Atlantic Ocean is noteworthy. The following species grow attached in Florida and the West Indies, where they are very common: *Turbinaria turbinata* (L.) Kuntze, *Centroceras clavulatum* (C. Ag.) Mont., and *Dictyosphaeria cavernosa* (Forssk.) Børg. These species were reported from North Carolina by Blomquist and Pyron (1943) following an influx of drifting seaweeds associated with a hurricane that swept here from the West Indies. They were not found to be a part of the flora of the reefs during this investigation. The high proportion of tropical species on the reefs is no doubt related to the warm currents from the tropics, but some of the southern species do not find conditions favorable to come in and grow and cannot, therefore, be considered a part of the flora of the Carolinas. Also noteworthy is the presence of tropical species on the reefs of the Carolinas that are not reported from the Caribbean Sea by Taylor (1942). Some of the more common of these species are *Cladophora pellucida*, two species of *Dictyopteris* (*D. polypodioides* and *D. serrata*), *Zonaria Tournefortii*, *Amphiroa brasiliana*, *Rhodymenia Palmetta*, and *Nitophyllum medium*.

GENERAL SUMMARY

1. The plants and animals on the reefs lying off the coasts of the Carolinas have been collected at depths of 12 to 55 ft.

2. A hundred two species and two varieties of algae (two new varieties) and 300 species of animals were taken or recorded (some fishes).

3. Of the algae 29 were from shallow water (12–20 ft.), 26 from deep water

(40–55 ft.) and 49 from both shallow and deep water. Of these 64 were oceanic and 40 were estuarine and oceanic.

4. In their latitudinal range 56 species of algae were distributed from North Carolina southward, 29 north and south, and 11 have been found in the Carolinas only. The identity and distribution of 6 species are unknown. Twenty-three are reported for the first time from the Carolinas.

5. A general description of the reefs and their flora is presented. The flora of the reefs is described in relationship to the flora of the near-by estuaries, and a general classification is given of the algal habitats in the Carolinas.

6. Animals were mostly of southern-ranging species (167), but some (63) had wide ranges along the Atlantic coast, and a few (18) ranged toward the north.

7. In depth 102 species of animals came from deep water, 96 from shallow, and 102 from both.

8. About a third of the species of animals were found off the Carolina coasts for the first time.

9. The reefs are mostly made of Trent Marl. On this, algae form a thick coating and in crevices and the flat sandy bottom associated with some of the deep Trent Marl, sand is loosely compacted by calcareous deposits from algae and shells of molluscs, but on the whole algae contribute little as reef builders.

10. Sessile snails and tubicolous annelids are the chief reef builders. Their shells (Figs. 1, 2) form coatings 4–5 inches thick and are being added to year after year. Two clams (*Saxicava*, *Lithophaga*) bore into the reefs and the shells of oysters and other molluscs add to them.

11. Though none of the reefs studied was more than ten miles offshore and still several miles from the Gulf Stream, the flora and fauna were predominantly southern in their relationships. Though madreporian and gorgonian corals were present, they were apparently not as a rule significant in adding to the reefs.

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